Welcome to STN International! Enter x:x

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Welcome to STN International
                 Web Page for STN Seminar Schedule - N. America
NEWS
      2
NEWS
         APR 02
                 CAS Registry Number Crossover Limits Increased to
                 500,000 in Key STN Databases
NEWS
      3
        APR 02
                 PATDPAFULL: Application and priority number formats
                 enhanced
NEWS
         APR 02
                 DWPI: New display format ALLSTR available
NEWS
         APR 02
                 New Thesaurus Added to Derwent Databases for Smooth
                 Sailing through U.S. Patent Codes
                 EMBASE Adds Unique Records from MEDLINE, Expanding
NEWS
        APR 02
                 Coverage back to 1948
NEWS
        APR 07
                 50,000 World Traditional Medicine (WTM) Patents Now
                 Available in CAplus
NEWS
      8
        APR 07
                 MEDLINE Coverage Is Extended Back to 1947
NEWS
      9
         JUN 16
                 WPI First View (File WPIFV) will no longer be
                 available after July 30, 2010
NEWS 10
         JUN 18
                 DWPI: New coverage - French Granted Patents
NEWS 11
         JUN 18
                 CAS and FIZ Karlsruhe announce plans for a new
                 STN platform
         JUN 18
NEWS 12
                 IPC codes have been added to the INSPEC backfile
                 (1969-2009)
NEWS 13
         JUN 21
                 Removal of Pre-IPC 8 data fields streamline displays
                 in CA/CAplus, CASREACT, and MARPAT
NEWS 14
         JUN 21
                 Access an additional 1.8 million records exclusively
                 enhanced with 1.9 million CAS Registry Numbers --
                 EMBASE Classic on STN
NEWS 15
         JUN 28
                 Introducing "CAS Chemistry Research Report": 40 Years
                 of Biofuel Research Reveal China Now Atop U.S. in
                 Patenting and Commercialization of Bioethanol
NEWS 16
         JUN 29
                 Enhanced Batch Search Options in DGENE, USGENE,
                 and PCTGEN
         JUL 19
NEWS 17
                 Enhancement of citation information in INPADOC
                 databases provides new, more efficient competitor
                 analyses
NEWS 18
         JUL 26
                 CAS coverage of global patent authorities has
                 expanded to 61 with the addition of Costa Rica
NEWS 19
         SEP 15
                 MEDLINE Cited References provide additional
                 revelant records with no additional searching.
NEWS 20
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                 Removal of Pre-IPC 8 data fields streamlines
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NEWS 21
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                 Increase your retrieval consistency with new formats or
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NEWS 23
         OCT 21
                 CA/CAplus kind code changes for Chinese patents
                 increase consistency, save time
NEWS 24
        OCT 22
                 New version of STN Viewer preserves custom
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highlighting of terms when patent documents are saved in .rtf format

NEWS 25 OCT 28 INPADOCDB/INPAFAMDB: Enhancements to the US national patent classification.

NEWS 26 NOV 03 New format for Korean patent application numbers in CA/CAplus increases consistency, saves time.

NEWS 27 NOV 04 Selected STN databases scheduled for removal on December 31, 2010

NEWS EXPRESS FEBRUARY 15 10 CURRENT WINDOWS VERSION IS V8.4.2, AND CURRENT DISCOVER FILE IS DATED 07 JULY 2010.

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FULL ESTIMATED COST

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62 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view search error messages that display as 0* with SET DETAIL OFF.

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 - 0* FILE ANTE
 - 0* FILE AQUALINE
 - 0* FILE BIOENG
 - 2* FILE BIOTECHABS
 - 2* FILE BIOTECHDS
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 - 0* FILE CEABA-VTB
 - 0* FILE CIN
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 - 0* FILE FOMAD
 - 0* FILE FROSTI
 - 0* FILE FSTA
 - 1 FILE IFIPAT
 - 0* FILE KOSMET
 - 0* FILE NTIS

1* FILE PASCAL

51 FILES SEARCHED...

- 4 FILE USPATFULL
- 1* FILE WATER
- 3 FILE WPIDS
- 3 FILE WPINDEX

8 FILES HAVE ONE OR MORE ANSWERS, 62 FILES SEARCHED IN STNINDEX

L1 QUE PERISTALTIC (P) ROL? AND CULTUR? (P) ALGA?

=> file biotechabs biotechds ifipat pascal uspatfull water
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SESSION
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FILE 'BIOTECHABS' ACCESS NOT AUTHORIZED

FILE 'BIOTECHDS' ENTERED AT 19:27:57 ON 09 NOV 2010 COPYRIGHT (C) 2010 THOMSON REUTERS

FILE 'IFIPAT' ENTERED AT 19:27:57 ON 09 NOV 2010 COPYRIGHT (C) 2010 IFI CLAIMS(R) Patent Services (IFI)

FILE 'PASCAL' ENTERED AT 19:27:57 ON 09 NOV 2010 Any reproduction or dissemination in part or in full, by means of any process and on any support whatsoever is prohibited without the prior written agreement of INIST-CNRS. COPYRIGHT (C) 2010 INIST-CNRS. All rights reserved.

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FILE 'WATER' ENTERED AT 19:27:57 ON 09 NOV 2010 COPYRIGHT (C) 2010 Cambridge Scientific Abstracts (CSA)

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FIELD CODE - 'AND' OPERATOR ASSUMED 'ERISTALTIC (P) ROL?'

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH

FIELD CODE - 'AND' OPERATOR ASSUMED 'CULTUR? (P) ALGA?'

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PROXIMITY OPERATOR ASSUMED 'CULTUR? (P) ALGA?'

LOUID OPERATOR ASSUMED 'CULTUR? (P) ALGA?'

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=> d 13 1-6

L3 ANSWER 1 OF 6 USPATFULL on STN

AN 2009:304349 USPATFULL

TI DEVICE FOR SPRAYING A COSMETIC COMPOSITION

IN ARNAUD, Pascal, L'Hay Les Roses, FRANCE COLLETTE, Annick, St. Maur Des Fosses, FRANCE

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BEAUMARD, Sophie, Villejuif, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
       TIERCE, Pascal, Bondues, FRANCE
       L'Oreal S.A., Paris, FRANCE (non-U.S. corporation)
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       US 20090272316
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DT
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FS
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INCL
       INCLM: 118/300.000
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       NCLM: 118/300.000
              B05B0007-00 [I,A]
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       BEAUMARD, Sophie, Villejuif, FRANCE
       ROULIER, Veronique, La Varenne St Hilaire, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
       TIERCE, Pascal, Bondues, FRANCE
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      Closed system bioreactor apparatus for bio-diesel producing system for
      producing bio-diesel from algae, comprises flexible tube(s),
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         closed system bioreactor apparatus and alga culture
         for biological diesel producing system and biological diesel
         production
ΑU
      SEARS J T
      SUNSOURCE IND
PA
PΙ
      US 20070048859 1 Mar 2007
ΑI
      US 2006-510442 24 Aug 2006
PRAI
      US 2006-510442 24 Aug 2006; US 2005-711316 25 Aug 2005
DT
      Patent
LA
      English
      WPI: 2007-387524 [36]
OS
L3
      ANSWER 4 OF 6 BIOTECHDS COPYRIGHT 2010 THOMSON REUTERS on STN DUPLICATE
      2007-13331 BIOTECHDS
ΑN
ΤТ
      Culturing algae comprises placing algae in
      aqueous medium in a closed system bioreactor, exposing the algae
```

to sunlight, and culturing the algae under conditions allowing algal reproduction and growth; involving cell culture of alga in a photoreactor useful for diesel production SEARS J T ΑU SUNSOURCE IND PA US 20070048848 1 Mar 2007 US 2006-510148 24 Aug 2006 PRAI US 2006-510148 24 Aug 2006; US 2005-711316 25 Aug 2005 Patent LA English WPI: 2007-387522 [36] ANSWER 5 OF 6 PASCAL COPYRIGHT 2010 INIST-CNRS. ALL RIGHTS RESERVED. on STN 1999-0122092 PASCAL Copyright .COPYRGT. 1999 INIST-CNRS. All rights reserved. CP The shear stress of microalgal cell suspensions (Tetraselmis suecica) in TIEN JAOUEN P.; VANDANJON L.; QUEMENEUR F. ΑU CS ISOMer, Institut des Substances et Organismes de la Mer, Centre de Recherche et de Transfert de Technologie, Laboratoire de Genie des Procede Boulevard de l'Universite, BP 406, 44 602 Saint-Nazaire, France SO Bioresource technology, (1999), 68(2), 149-154, 14 refs.

DT Journal

PΙ

ΑI

DT

OS

L3

ΑN

- Analytic BL
- CYUnited Kingdom
- LA English
- ΑV INIST-18769, 354000074072090060
- ANSWER 6 OF 6 WATER COPYRIGHT 2010 CSA on STN L3
- 2004155548 WATER ΑN

ISSN: 0960-8524

- DN 8205175
- ΤI Cyanobacterial Blooms: Carbon and Nitrogen Limitation have Opposite Effects on the Buoyance of Oscillatori
- ΑU Klemer, AR; Feuillade, J; Feuillade, M
- CS State Univ. of New York Coll. at Purchase
- SO Science Vol 215, No 4540, p 1629-1631, March 26, 1982. 2 Fig, 27 Ref.

=> d 13 5 ab

- L3 ANSWER 5 OF 6 PASCAL COPYRIGHT 2010 INIST-CNRS. ALL RIGHTS RESERVED. on
- AΒ A widely used technique for cell harvesting is crossflow microfiltration. The relative fragility of microalgae requires choosing a suitable pumping system. Tetraselmis suecica, which is a very mobile alga, is an interesting model for this study. The extent of damage observed in various pumps was measured as a function of the number of passes of the algal suspension through the pumps in a closed loop. A loss of microalgal motility could occur with either centrifugal or rotary vane positive displacement pumps. On the contrary, electronic microscope observation revealed that, during concentration using an eccentric rotor pump or a peristaltic pump, microalgae were not damaged.
- => s 13 and roller? L44 L3 AND ROLLER?
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      Closed system bioreactor apparatus for bio-diesel producing system for
      producing bio-diesel from algae, comprises flexible tube(s),
      peristaltic roller(s), and thermal barrier;
         closed system bioreactor apparatus and alga culture
         for biological diesel producing system and biological diesel
         production
ΑU
      SEARS J T
      SUNSOURCE IND
PA
      US 20070048859 1 Mar 2007
PI
ΑI
      US 2006-510442 24 Aug 2006
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      US 2006-510442 24 Aug 2006; US 2005-711316 25 Aug 2005
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OS
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T.4
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TΙ
      Culturing algae comprises placing algae in
      aqueous medium in a closed system bioreactor, exposing the algae
      to sunlight, and culturing the algae under conditions
      allowing algal reproduction and growth;
         involving cell culture of alga in a photoreactor
         useful for diesel production
ΑU
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      SUNSOURCE IND
PA
PΤ
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      US 2006-510148 24 Aug 2006
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      English
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       DEVICE FOR SPRAYING A COSMETIC COMPOSITION
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      Culturing algae comprises placing algae in aqueous
      medium in a closed system bioreactor, exposing the algae to
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      algal reproduction and growth;
         involving cell culture of alga in a photoreactor useful for
         diesel production
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      SEARS J T
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      SUNSOURCE IND
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      US 2006-510148 24 Aug 2006
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      Last Updated on STN: 12 Apr 2007
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AN
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      Last Updated on STN: 12 Apr 2007
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ΑN
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       Tzipori, Saul, Shrewsbury, MA, UNITED STATES
       Stacey, Gary, Marshfield, MA, UNITED STATES
       TRUSTEES OF TUFTS COLLEGE, Medford, MA, UNITED STATES (U.S. corporation)
PA
       HAEMONETICS CORPORATION, Braintree, MA, UNITED STATES (U.S. corporation)
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       Enzymes
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ΙN
       Wales, Melinda E., Bryan, TX, UNITED STATES
       Rawlins, James, Hattiesburg, MS, UNITED STATES
       Cipi, Pirro, Hattiesburg, MS, UNITED STATES
       Williams, Eric, Petal, MS, UNITED STATES
       Carvajal, Juan Carlo, Austin, TX, UNITED STATES
       REACTIVE SURFACES, LTD., Austin, TX, UNITED STATES (U.S. corporation)
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       US 2008-57705P
                               20080530 (61)
       US 2008-58025P
                               20080602 (61)
       US 2003-485234P
                               20030703 (60)
       US 2007-976676P
                               20071001 (60)
       US 2002-409102P
                               20020909 (60)
DT
       Utility
FS
       APPLICATION
LN.CNT 37946
       INCLM: 521/055.000
INCL
       INCLS: 524/017.000
NCL
              521/055.000
       NCLM:
       NCLS:
              524/017.000
IC
       IPCI
              C09D0007-12 [I,A]; C09J0011-08 [I,A]; C09J0011-00 [I,C*]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 7 OF 36 USPATFULL on STN
AN
       2010:179163 USPATFULL
ΤI
       PHOTOBIOREACTOR SYSTEMS
ΙN
       Schuring, Christopher S., Penryn, CA, UNITED STATES
       McCue, J. Kyle, San Jose, CA, UNITED STATES
PΙ
       US 20100159579
                           A1 20100624
                           A1 20091020 (12)
ΑI
       US 2009-582697
                               20081020 (61)
PRAI
       US 2008-106962P
DT
       Utility
FS
       APPLICATION
LN.CNT 602
INCL
       INCLM: 435/292.100
NCL
       NCLM: 435/292.100
IC
       IPCI
              C12M0001-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 8 OF 36 USPATFULL on STN
AN
       2009:304349 USPATFULL
ΤI
       DEVICE FOR SPRAYING A COSMETIC COMPOSITION
ΙN
       ARNAUD, Pascal, L'Hay Les Roses, FRANCE
       COLLETTE, Annick, St. Maur Des Fosses, FRANCE
       BEAUMARD, Sophie, Villejuif, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
       TIERCE, Pascal, Bondues, FRANCE
       L'Oreal S.A., Paris, FRANCE (non-U.S. corporation)
PA
PΙ
       US 20090272316
                           A1 20091105
       US 2009-369854
                           A1
                               20090212 (12)
AΙ
PRAI
       FR 2008-50923
                                20080213
       US 2008-71061P
                               20080410 (61)
DT
       Utility
FS
       APPLICATION
LN.CNT 1695
       INCLM: 118/300.000
INCL
NCL
       NCLM:
              118/300.000
IC
       IPCI
              B05B0007-00 [I,A]
       IPCR
              B05B0007-00 [I,C]; B05B0007-00 [I,A]
L9
     ANSWER 9 OF 36 USPATFULL on STN
ΑN
       2009:288768 USPATFULL
ΤТ
       PRODUCTION OF SILVER SULFATE GRAINS USING A FLUORINATED ADDITIVE
```

```
TM
       Sandford, David W., Rochester, NY, UNITED STATES
       Blanton, Thomas N., Rochester, NY, UNITED STATES
       US 20090258984
                           Α1
PΤ
                               20091015
       US 7655212
                           В2
                               20100202
       US 2008-101237
                           A1 20080411 (12)
AΙ
DT
       Utility
FS
       APPLICATION
LN.CNT 2722
INCL
       INCLM: 524/403.000
       INCLS: 423/544.000; 252/182.110; 252/182.320
              423/544.000; 524/403.000
NCL
              524/403.000; 524/423.000; 252/182.110; 252/182.320
       NCLS:
IC
       IPCI
              C08K0003-10 [I,A]; C08K0003-00 [I,C*]; C01B0017-96 [I,A];
              C01B0017-00 [I,C*]; C09K0003-00 [I,A]
       IPCI-2 C01G0005-00 [I,A]; C08K0003-00 [I,A]
       IPCR
              C01G0005-00 [I,C]; C01G0005-00 [I,A]; C08K0003-00 [I,C];
              C08K0003-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 10 OF 36 USPATFULL on STN
ΑN
       2009:288004 USPATFULL
ΤI
       PRODUCTION OF SILVER SULFATE GRAINS USING CARBOXYLIC ACID ADDITIVES
ΙN
       Sandford, David W., Rochester, NY, UNITED STATES
       Blanton, Thomas N., Rochester, NY, UNITED STATES
PΙ
       US 20090258218
                           A1 20091015
ΑI
       US 2008-101249
                           A1 20080411 (12)
DT
       Utility
       APPLICATION
FS
LN.CNT 2193
TNCL
       INCLM: 428/327.000
       INCLS: 423/561.100; 428/402.000
NCL
       NCLM: 428/327.000
       NCLS:
              423/561.100; 428/402.000
IC
       IPCI
              B32B0005-16 [I,A]; H01M0004-58 [I,A]
       IPCR
              B32B0005-16 [I,C]; B32B0005-16 [I,A]; H01M0004-58 [I,C];
              H01M0004-58 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 11 OF 36 USPATFULL on STN
       2009:230118 USPATFULL
AN
ΤI
       DEVICE FOR SPRAYING A COSMETIC COMPOSITION
IN
       ARNAUD, Pascal, L'Hay Les Roses, FRANCE
       BEAUMARD, Sophie, Villejuif, FRANCE
       ROULIER, Veronique, La Varenne St Hilaire, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
       TIERCE, Pascal, Bondues, FRANCE
       L'Oreal S.A., Paris, FRANCE (non-U.S. corporation)
PA
РΤ
       US 20090206174
                           A1 20090820
                               20090212 (12)
ΑI
       US 2009-370217
                           Α1
PRAI
       FR 2008-50922
                                20080213
       US 2008-71060P
                               20080410 (61)
DT
       Utility
FS
       APPLICATION
LN.CNT 1717
INCL
       INCLM: 239/102.200
NCL
       NCLM:
              239/102.200
IC
       IPCI
              B05B0001-08 [I,A]; B05B0001-02 [I,C*]
              B05B0001-02 [I,C]; B05B0001-08 [I,A]
       IPCR
L9
     ANSWER 12 OF 36 USPATFULL on STN
ΑN
       2009:153195 USPATFULL
```

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CRANIUM APPARATUS
TΤ
TN
       FALLAH, AFSHIN AL, San Diego, CA, UNITED STATES
РΤ
       US 20090138040
                           A1 20090528
       US 2008-325109
                           A1 20081128 (12)
AΙ
PRAI
       US 2007-990617P
                                20071128 (60)
       Utility
DT
FS
       APPLICATION
LN.CNT 627
       INCLM: 606/204.150
NCL
       NCLM:
             606/204.150
IC
              A61F0005-00 [I,A]
       IPCI
       IPCR
              A61F0005-00 [I,C]; A61F0005-00 [I,A]
L9
     ANSWER 13 OF 36 USPATFULL on STN
ΑN
       2008:140437 USPATFULL
ΤI
       System and method for dispensing an aseptic food product from a
       container
       Gehl, John P., Nashotah, WI, UNITED STATES
ΙN
                           A1 20080529
ΡI
       US 20080121662
                           A1
ΑI
       US 2006-605097
                               20061128 (11)
DT
       Utility
FS
       APPLICATION
LN.CNT 952
       INCLM: 222/214.000
INCL
       INCLS: 222/146.200
NCL
       NCLM:
              222/214.000
       NCLS:
              222/146.200
IC
       IPCI
              B65D0037-00 [I,A]; B67D0005-62 [I,A]
       IPCR
              B65D0037-00 [I,C]; B65D0037-00 [I,A]; B67D0007-80 [I,C*];
              B67D0007-80 [I,A]
L9
     ANSWER 14 OF 36 USPATFULL on STN
ΑN
       2008:111413 USPATFULL
ΤI
       Food Fortification with Polyunsaturated Fatty Acids
ΙN
       Subramanian, Srinivasan, Broomfield, CO, UNITED STATES
       Connolly, Brian, Broomfield, CO, UNITED STATES
       Crandell, Michelle, Boulder, CO, UNITED STATES
       Abril, Jesus Ruben, Westminster, CO, UNITED STATES
       MARTEK BIOSCIENCES CORPORATION, Columbia, MD, UNITED STATES, 21045 (U.S.
PA
       corporation)
PΙ
       US 20080096964
                           Α1
                               20080424
       US 2007-845575
                           A1 20070827 (11)
ΑТ
PRAI
       US 2006-823599P
                                20060825 (60)
DT
       Utility
FS
       APPLICATION
LN.CNT 1720
INCL
       INCLM: 514/560.000
       INCLS: 426/289.000; 426/302.000; 426/304.000; 426/309.000; 426/601.000;
              426/089.000
NCL
       NCLM:
              514/560.000
       NCLS:
              426/089.000; 426/289.000; 426/302.000; 426/304.000; 426/309.000;
              426/601.000
              A23P0001-08 [I,A]; A23D0007-00 [I,A]; A61K0031-202 [I,A];
IC
       IPCI
              A61K0031-185 [I,C*]
              A23P0001-08 [I,C]; A23P0001-08 [I,A]; A23D0007-00 [I,C];
       IPCR
              A23D0007-00 [I,A]; A61K0031-185 [I,C]; A61K0031-202 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 15 OF 36 USPATFULL on STN
1.9
AΝ
       2008:17838 USPATFULL
       METHOD AND APPARATUS FOR NONINVASIVE INTRADUCTAL FLUID DIAGNOSTIC SCREEN
TT
TN
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
```

```
Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
       Neomatrix, LLC, Irvine, CA, UNITED STATES, 92618 (U.S. corporation)
PA
PΙ
       US 20080015495
                           Α1
                               20080117
ΑI
       US 2007-775751
                           A1
                              20070710 (11)
       Continuation of Ser. No. US 2005-99295, filed on 5 Apr 2005, PENDING
RLI
       Continuation of Ser. No. US 2002-72538, filed on 8 Feb 2002, GRANTED,
       Pat. No. US 6875184 Continuation-in-part of Ser. No. US 2001-870402,
       filed on 30 May 2001, GRANTED, Pat. No. US 6866994
DT
       Utility
FS
       APPLICATION
LN.CNT 2072
       INCLM: 604/074.000
INCL
NCL
       NCLM:
              604/074.000
IC
       IPCI
              A61M0001-06 [I,A]
              A61M0001-06 [I,C]; A61M0001-06 [I,A]
       IPCR
L9
     ANSWER 16 OF 36 USPATFULL on STN
ΑN
       2008:17812
                  USPATFULL
ΤI
       DISPOSABLE PATIENT INTERFACE FOR INTRADUCTAL FLUID ASPIRATION SYSTEM
ΙN
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PA
       Neomatrix, LLC, Irvine, CA, UNITED STATES (U.S. corporation)
                           A1 20080117
PΙ
       US 20080015469
                           A1 20070710 (11)
       US 2007-775768
ΑТ
       Continuation of Ser. No. US 2002-209210, filed on 30 Jul 2002, PENDING
RLI
       Continuation-in-part of Ser. No. US 2002-72546, filed on 8 Feb 2002,
       GRANTED, Pat. No. US 6676610 Continuation-in-part of Ser. No. US
       2001-870402, filed on 30 May 2001, GRANTED, Pat. No. US 6866994
DT
       Utility
FS
       APPLICATION
LN.CNT 2365
INCL
       INCLM: 600/573.000
NCL
       NCLM:
             600/573.000
IC
       IPCI
              A61B0010-02 [I,A]
       IPCR
              A61B0010-02 [I,C]; A61B0010-02 [I,A]
L9
     ANSWER 17 OF 36 USPATFULL on STN
ΑN
       2007:124720 USPATFULL
TΙ
       ELECTROCHEMICAL ION EXCHANGE TREATMENT OF FLUIDS
ΙN
       Nyberg, Eric David, Belmont, CA, UNITED STATES
       Vogdes, Christine Ellen, Sunnyvale, CA, UNITED STATES
       Holmes, James Crawford, San Carlos, CA, UNITED STATES
       Janah, Ashok Kumar, San Francisco, CA, UNITED STATES
PA
       Pionetics Corporation (U.S. corporation)
PΙ
       US 20070108056
                           A1 20070517
                           A1
ΑI
       US 2006-539596
                               20061006 (11)
       US 2005-724456P
                               20051006 (60)
PRAI
       US 2006-831703P
                               20060717 (60)
DT
       Utility
       APPLICATION
FS
LN.CNT 3982
INCL
       INCLM: 204/554.000
       INCLS: 204/660.000
NCL
       NCLM:
             204/554.000
       NCLS:
              204/660.000
IC
       IPCI
              B03C0005-00 [I,A]; B03C0005-02 [I,A]
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B03C0005-00 [I,C]; B03C0005-00 [I,A]; B03C0005-02 [I,A]
       TPCR
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 18 OF 36 USPATFULL on STN
L9
       2007:55917 USPATFULL
ΑN
TΙ
       Closed system bioreactor apparatus
ΙN
       Sears, James T., Boulder, CO, UNITED STATES
       SUNSOURCE INDUSTRIES (U.S. corporation)
PA
PΙ
       US 20070048859
                           A1 20070301
       US 2006-510442
                           A1 20060824 (11)
AΙ
       US 2005-711316P
                               20050825 (60)
PRAI
       US 2005-733569P
                               20051104 (60)
       US 2005-740855P
                               20051130 (60)
       US 2006-757587P
                               20060110 (60)
                               20060630 (60)
       US 2006-818102P
DT
       Utility
FS
       APPLICATION
LN.CNT 1648
       INCLM: 435/289.100
INCL
NCL
       NCLM:
             435/289.100
IC
       IPCI
              C12M0003-00 [I,A]
       IPCR
              C12M0003-00 [I,C]; C12M0003-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 19 OF 36 USPATFULL on STN
L9
ΑN
       2007:55906 USPATFULL
       Method, apparatus and system for biodiesel production from algae
ΤТ
ΙN
       Sears, James T., Boulder, CO, UNITED STATES
PA
       SUNSOURCE INDUSTRIES (U.S. corporation)
                          A1 20070301
PΙ
       US 20070048848
       US 2006-510148
                           A1 20060824 (11)
ΑТ
PRAI
       US 2005-711316P
                               20050825 (60)
       US 2005-733569P
                               20051104 (60)
       US 2005-740855P
                               20051130 (60)
       US 2006-757587P
                               20060110 (60)
       US 2006-818102P
                               20060630 (60)
DT
       Utility
       APPLICATION
FS
LN.CNT 1738
       INCLM: 435/134.000
INCL
       INCLS: 435/257.100; 554/174.000
NCL
       NCLM:
             435/134.000
       NCLS:
             435/257.100; 554/174.000
TC
       IPCI
              C12P0007-64 [I,A]; C12N0001-12 [I,A]; C07C0051-43 [I,A];
              C07C0051-42 [I,C*]
              C12P0007-64 [I,C]; C12P0007-64 [I,A]; C07C0051-42 [I,C];
       IPCR
              C07C0051-43 [I,A]; C12N0001-12 [I,C]; C12N0001-12 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 20 OF 36 USPATFULL on STN
L9
ΑN
       2006:254042 USPATFULL
ΤI
       Foam Composite
IN
       Thomson, Timothy, 124 Main Street, West Newbury, MA, UNITED STATES
       01985
       Hydrophilix, LLC, West Newbury, MA, UNITED STATES (U.S. corporation)
PA
РΤ
       US 20060216492
                           A1 20060928
ΑI
       US 2006-277103
                           Α1
                              20060321 (11)
RLI
       Continuation of Ser. No. US 2005-230964, filed on 20 Sep 2005, GRANTED,
       Pat. No. US 7048966 Division of Ser. No. US 2003-421283, filed on 23 Apr
       2003, GRANTED, Pat. No. US 6991848 Continuation of Ser. No. US
       2001-823129, filed on 30 Mar 2001, ABANDONED Continuation-in-part of
       Ser. No. US 2000-540099, filed on 31 Mar 2000, GRANTED, Pat. No. US
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6617014
       Utility
DT
FS
       APPLICATION
LN.CNT 1535
       INCLM: 428/304.400
INCL
NCL
       NCLM:
             428/304.400
IC
       IPCI
              B32B0003-26 [I,A]
       IPCR
              B32B0003-26 [I,C]; B32B0003-26 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 21 OF 36 USPATFULL on STN
       2006:15583 USPATFULL
ΑN
TΙ
       Foam composite
ΙN
       Thomson, Timothy, West Newbury, MA, UNITED STATES
PΑ
       Hydrophilix, LLC, West Newbury, MA, UNITED STATES, 01985 (U.S.
       corporation)
       US 20060013963
                           Α1
                               20060119
PΙ
       US 7048966
                           В2
                               20060523
       US 2005-230964
                               20050920 (11)
ΑI
                           Α1
       Division of Ser. No. US 2003-421283, filed on 23 Apr 2003, PENDING
RLI
       Continuation of Ser. No. US 2001-823129, filed on 30 Mar 2001, ABANDONED
       Continuation-in-part of Ser. No. US 2000-540099, filed on 31 Mar 2000,
       GRANTED, Pat. No. US 6617014
DT
       Utility
FS
       APPLICATION
LN.CNT 1619
INCL
       INCLM: 427/487.000
       INCLS: 521/065.000; 427/512.000
NCL
       NCLM:
             427/244.000; 427/487.000
             427/243.000; 427/373.000; 427/512.000; 521/065.000
       NCLS:
              C08J0009-28 [I,A]; C08J0009-00 [I,C*]; C08F0002-46 [I,A]
IC
       IPCI
       IPCI-2 B05D0005-00 [I,A]
              C08J0009-00 [I,C]; C08J0009-28 [I,A]; C08F0002-46 [I,C];
              C08F0002-46 [I,A]; B05D0005-00 [I,A]; B05D0005-00 [I,C]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 22 OF 36 USPATFULL on STN
AN
       2005:247213 USPATFULL
ΤI
       Combination continuous/batch fermentation processes
       Pilkington, Phyllis Heather, London, CANADA
       Mensour, Normand Anthony, Ancaster, CANADA
PΙ
       US 20050214408
                           A1 20050929
ΑТ
       US 2005-98688
                           A1 20050405 (11)
RT.T
       Continuation-in-part of Ser. No. US 2002-175351, filed on 20 Jun 2002,
       PENDING
       Utility
DT
FS
       APPLICATION
LN.CNT 5429
       INCLM: 426/016.000
INCL
       INCLS: 435/161.000
NCL
       NCLM:
             426/016.000
       NCLS:
              435/161.000
IPC
       [7]
              C12P0007-06 [ICM, 7]; C12P0007-02 [ICM, 7, C*]
       IPCI
              C12C0011-00 [I,C*]; C12C0011-07 [I,A]; C12C0011-09 [I,A];
       IPCR
              C12P0007-02 [I,C*]; C12P0007-06 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 23 OF 36 USPATFULL on STN
1.9
       2005:197327 USPATFULL
ΑN
TΤ
       Method and apparatus for noninvasive intraductal fluid diagnostic screen
TN
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
```

```
Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
       NEOMATRIX, LLC (U.S. corporation)
PA
PΙ
       US 20050171471
                           A1 20050804
       US 7468043
                           В2
                               20081223
ΑI
       US 2005-99295
                           A1 20050405 (11)
RLI
       Continuation of Ser. No. US 2002-72538, filed on 8 Feb 2002, GRANTED,
       Pat. No. US 6875184 Continuation-in-part of Ser. No. US 2001-870402,
       filed on 30 May 2001, GRANTED, Pat. No. US 6866994
DT
       Utility
FS
       APPLICATION
LN.CNT 2068
       INCLM: 604/097.010
INCL
       NCLM: 600/573.000; 604/097.010
NCL
       NCLS: 604/074.000
IPC
       [7]
       IPCI
              A61M0029-00 [ICM, 7]
       IPCI-2 A61B0005-00 [I,A]; B65D0081-00 [I,A]
       IPCR
              A61B0005-00 [I,C]; A61B0005-00 [I,A]; A61B0010-00 [I,C*];
              A61B0010-00 [I,A]; A61B0010-02 [N,C*]; A61B0010-02 [N,A];
              A61M0001-06 [N,C*]; A61M0001-06 [N,A]; B65D0081-00 [I,C];
              B65D0081-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 24 OF 36 USPATFULL on STN
1.9
AN
       2004:234130 USPATFULL
ΤI
       Method of noninvasively obtaining intraductal fluid
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
ΤN
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PΙ
                           A1 20040916
       US 20040181205
       US 6899696
                           B2 20050531
ΑI
       US 2004-811762
                           A1 20040329 (10)
       Continuation of Ser. No. US 2002-72539, filed on 8 Feb 2002, GRANTED,
RLI
       Pat. No. US 6712785 Continuation-in-part of Ser. No. US 2001-870402,
       filed on 30 May 2001, PENDING
DT
       Utility
FS
       APPLICATION
LN.CNT 2018
INCL
       INCLM: 604/500.000
              604/074.000; 604/500.000
NCL
       NCLM:
              424/537.000; 435/283.100; 604/076.000; 604/118.000; 604/120.000
       NCLS:
IPC
       [7]
              G01N0033-574 [ICM, 7]; A61M0031-00 [ICS, 7]
       IPCI
       IPCI-2 A61M0001-00 [ICM, 7]; A61M0001-06 [ICS, 7]; A61K0035-12 [ICS, 7]
              A61B0010-00 [I,C*]; A61B0010-00 [I,A]; A61B0010-02 [N,C*];
       IPCR
              A61B0010-02 [N,A]; A61M0001-06 [N,C*]; A61M0001-06 [N,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 25 OF 36 USPATFULL on STN
       2003:288424 USPATFULL
AN
ΤI
       Foam composite
ΙN
       Thomson, Timothy, West Newbury, MA, UNITED STATES
PA
       Hydrophilix (U.S. corporation)
PΙ
       US 20030203182
                           A1 20031030
       US 6991848
                           В2
                               20060131
       US 2003-421283
                           A1 20030423 (10)
ΑТ
RLI
       Continuation of Ser. No. US 2001-823129, filed on 30 Mar 2001, ABANDONED
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Continuation-in-part of Ser. No. US 2000-540099, filed on 31 Mar 2000,
       GRANTED, Pat. No. US 6617014
       Utility
DT
       APPLICATION
FS
LN.CNT 1764
INCL
       INCLM: 428/315.500
       INCLS: 428/317.900; 428/316.600; 428/308.400
NCL
             428/316.600; 428/315.500
       NCLS:
              428/306.600; 428/308.400; 428/315.700; 428/317.900
IPC
       [7]
       IPCI
              B32B0003-26 [ICM, 7]
       IPCI-2 B32B0003-26 [I,A]
       IPCR
              B32B0005-18 [I,C*]; B32B0005-18 [I,A]; C08J0009-00 [I,C*];
              C08J0009-42 [I,A]; B32B0003-26 [I,A]; B32B0003-26 [I,C]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 26 OF 36 USPATFULL on STN
T.9
       2003:248494 USPATFULL
ΑN
       METHOD AND APPARATUS FOR MONITORING A FLUID SYSTEM
ΤI
ΙN
       Staffler, Michael, Furstenfeldbruck, GERMANY, FEDERAL REPUBLIC OF
PΙ
       US 20030173272
                           A1
                               20030918
       US 6623630
                           В2
                                20030923
AΙ
       US 2002-99055
                           A1
                               20020313 (10)
DT
       Utility
FS
       APPLICATION
LN.CNT 696
INCL
       INCLM: 210/087.000
       INCLS: 210/198.200; 340/606.000; 340/609.000
NCL
       NCLM: 210/087.000
       NCLS:
              073/061.560; 073/861.080; 073/861.410; 210/198.200; 340/605.000;
              340/609.000; 417/474.000; 340/606.000
IPC
       [7]
       IPCI
              B01D0017-12 [ICM, 7]; B01D0017-00 [ICM, 7, C*]
       IPCI-2 B01D0017-12 [ICM,7]; B01D0017-00 [ICM,7,C*]; B01D0015-08 [ICS,7];
              G01F0001-56 [ICS, 7]
       IPCR
              G01F0001-20 [I,C*]; G01F0001-20 [I,A]; B01D0015-08 [I,C*];
              B01D0015-08 [I,A]; B01D0017-00 [I,C*]; B01D0017-12 [I,A];
              G01F0001-56 [I,C*]; G01F0001-56 [I,A]; G01M0003-02 [I,C*];
              G01M0003-02 [I,A]; G01N0030-00 [I,C*]; G01N0030-26 [I,A];
              G01N0030-32 [I,A]; G01N0030-36 [I,A]; G01N0030-64 [I,A];
              G01N0030-86 [I,A]
L9
     ANSWER 27 OF 36 USPATFULL on STN
ΑN
       2003:240273 USPATFULL
TΙ
       Foam composite
       Thomson, Timothy, West Newbury, MA, United States
TN
       Hydrophilix, LLC, Portland, ME, United States (U.S. corporation)
PA
PΙ
       US 6617014
                           B1 20030909
       US 2000-540099
ΑI
                                20000331 (9)
       Continuation-in-part of Ser. No. US 1999-387466, filed on 1 Sep 1999,
RLI
       now abandoned
DT
       Utility
FS
       GRANTED
LN.CNT 1405
       INCLM: 428/304.400
TNCL
       INCLS: 428/308.400; 428/309.900; 428/315.700; 428/316.600; 428/305.500;
              428/306.600; 428/319.300
NCL
       NCLM:
              428/304.400
       NCLS:
              428/305.500; 428/306.600; 428/308.400; 428/309.900; 428/315.700;
              428/316.600; 428/319.300
IPC
       [7]
       IPCI
              B32B0003-26 [ICM, 7]; B32B0003-06 [ICS, 7]
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IPCR
              B32B0003-06 [I,C*]; B32B0003-06 [I,A]; B32B0003-26 [I,C*];
              B32B0003-26 [I,A]
EXF
       428/304.4; 428/308.4; 428/309.9; 428/315.7; 428/316.6; 428/305.5;
       428/306.6; 428/319.3
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 28 OF 36 USPATFULL on STN
ΑN
       2003:219769 USPATFULL
ΤI
       Combination continuous/batch fermentation processes
       Pilkington, Phyllis Heather, London, CANADA
ΙN
       Mensour, Normand Anthony, London, CANADA
                           A1 20030814
PΤ
       US 20030153059
ΑI
       US 2002-175351
                           A1 20020620 (10)
PRAI
       US 2001-299153P
                               20010620 (60)
       US 2001-299186P
                               20010620 (60)
DТ
       Utility
       APPLICATION
FS
LN.CNT 6316
INCL
       INCLM: 435/161.000
       INCLS: 426/016.000
NCL
       NCLM:
             435/161.000
       NCLS:
             426/016.000
IPC
       [7]
       IPCI
              C12P0007-06 [ICM, 7]; C12P0007-02 [ICM, 7, C*]
       IPCR
              C12C0011-00 [I,C*]; C12C0011-07 [I,A]; C12C0011-09 [I,A];
              C12P0007-02 [I,C*]; C12P0007-06 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 29 OF 36 USPATFULL on STN
ΑN
       2003:107037 USPATFULL
ΤI
       Disposable patient interface for intraductal fluid aspiration system
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
ΤN
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PΙ
       US 20030073951
                           A1 20030417
       US 2002-209210
                           A1 20020730 (10)
ΑI
RLI
       Continuation-in-part of Ser. No. US 2002-72546, filed on 8 Feb 2002,
       PENDING Continuation-in-part of Ser. No. US 2001-870402, filed on 30 May
       2001, PENDING
DT
       Utility
FS
       APPLICATION
LN.CNT 2516
INCL
       INCLM: 604/073.000
NCL
       NCLM: 604/073.000
IPC
       [7]
       IPCI
              A61M0001-06 [ICM, 7]
       IPCR
              A61B0010-00 [I,C*]; A61B0010-00 [I,A]; A61B0010-02 [N,C*];
              A61B0010-02 [N,A]; A61M0001-06 [N,C*]; A61M0001-06 [N,A]
     ANSWER 30 OF 36 USPATFULL on STN
L9
ΑN
       2003:58166 USPATFULL
ΤI
       Method of noninvasively obtaining intraductal fluid
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
ΤN
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PΙ
       US 20030040734
                           A1 20030227
       US 6712785
                           B2 20040330
       US 2002-72539
                           A1 20020208 (10)
AΙ
```

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Continuation-in-part of Ser. No. US 2001-870402, filed on 30 May 2001,
RLI
       PENDING
       Utility
DT
       APPLICATION
FS
LN.CNT 2077
INCL
       INCLM: 604/514.000
       INCLS: 604/074.000
NCL
       NCLM:
              604/074.000; 604/514.000
       NCLS:
              424/537.000; 435/283.100; 604/076.000; 604/118.000; 604/120.000
IPC
       IPCI
              A61M0001-06 [ICM, 7]; A61M0031-00 [ICS, 7]
       IPCI-2 A61M0001-00 [ICM, 7]; A61M0001-06 [ICS, 7]; A61K0035-12 [ICS, 7]
              A61B0010-00 [I,C*]; A61B0010-00 [I,A]; A61B0010-02 [N,C*];
              A61B0010-02 [N,A]; A61M0001-06 [N,C*]; A61M0001-06 [N,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 31 OF 36 USPATFULL on STN
T.9
ΑN
       2002:323545 USPATFULL
ΤI
       Disposable patient interface for intraductal fluid aspiration system
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
ΙN
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PΙ
                                20021205
       US 20020183719
                            A1
                                20040113
       US 6676610
                            В2
       US 2002-72546
                                20020208 (10)
ΑI
                            A1
       Continuation-in-part of Ser. No. US 2001-870402, filed on 30 May 2001,
RLI
       PENDING
       Utility
DT
       APPLICATION
FS
LN.CNT 2069
       INCLM: 604/514.000
TNCL
       INCLS: 604/074.000
NCL
       NCLM: 600/573.000; 604/514.000
       NCLS:
             604/074.000; 604/317.000
IPC
       [71]
       IPCI
              A61M0001-06 [ICM, 7]; A61M0031-00 [ICS, 7]
       IPCI-2 A61B0005-00 [ICM, 7]
              A61B0010-00 [I,C*]; A61B0010-00 [I,A]; A61B0010-02 [N,C*];
              A61B0010-02 [N,A]; A61M0001-06 [N,C*]; A61M0001-06 [N,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 32 OF 36 USPATFULL on STN
       2002:323544 USPATFULL
ΑN
ΤI
       Disposable fluid loop for intraductal fluid aspiration system
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
ΙN
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PΙ
       US 20020183718
                            A1
                                20021205
       US 7575557
                            В2
                                20090818
                                20020208 (10)
       US 2002-72543
ΑI
                            A1
       Continuation-in-part of Ser. No. US 2001-870402, filed on 30 May 2001,
RLI
       PENDING
DT
       Utility
FS
       APPLICATION
LN.CNT 2056
       INCLM: 604/514.000
INCL
       INCLS: 604/074.000
NCL
       NCLM: 600/573.000; 604/514.000
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NCLS: 604/074.000
IPC
       [71]
              A61M0001-06 [ICM, 7]; A61M0031-00 [ICS, 7]
       IPCI
       IPCI-2 A61B0005-00 [I,A]; B65D0081-00 [I,A]
              A61B0005-00 [I,C]; A61B0005-00 [I,A]; A61B0010-00 [I,C*];
              A61B0010-00 [I,A]; A61B0010-02 [N,C*]; A61B0010-02 [N,A];
              A61M0001-06 [N,C*]; A61M0001-06 [N,A]; B65D0081-00 [I,C];
              B65D0081-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 33 OF 36 USPATFULL on STN
       2002:323543 USPATFULL
AN
TΙ
       Method and apparatus for noninvasive intraductal fluid diagnostic screen
IN
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PΙ
       US 20020183717
                           A1 20021205
       US 6875184
                           В2
                               20050405
ΑI
       US 2002-72538
                           A1
                               20020208 (10)
RLI
       Continuation-in-part of Ser. No. US 2001-870402, filed on 30 May 2001,
       PENDING
DT
       Utility
       APPLICATION
FS
LN.CNT 2147
INCL
       INCLM: 604/514.000
       INCLS: 604/074.000
NCL
       NCLM: 600/573.000; 604/514.000
       NCLS: 604/074.000; 604/313.000
IPC
       [7]
       IPCI
              A61M0001-06 [ICM, 7]
       IPCI-2 A61B0005-00 [ICM, 7]; B65D0081-00 [ICS, 7]
              A61B0010-00 [I,C*]; A61B0010-00 [I,A]; A61B0010-02 [N,C*];
              A61B0010-02 [N,A]; A61M0001-06 [N,C*]; A61M0001-06 [N,A]
L9
     ANSWER 34 OF 36 USPATFULL on STN
AN
       2002:322549 USPATFULL
ΤI
       Noninvasive intraductal fluid sampling device
       Morton, Kevin B., Mission Viejo, CA, UNITED STATES
ΤN
       Bare, Rex O., Lake Forest, CA, UNITED STATES
       Smith, Jeffrey C., Newport Beach, CA, UNITED STATES
       Payne, Timothy J., Santa Ana, CA, UNITED STATES
       Gleason, Paul, Laguna Niguel, CA, UNITED STATES
PΤ
       US 20020182713
                           A1
                               20021205
       US 6981950
                           В2
                               20060103
       US 2002-72537
                               20020208 (10)
AΙ
                           A1
       Continuation-in-part of Ser. No. US 2001-870402, filed on 30 May 2001,
RLI
       PENDING
DT
       Utility
FS
       APPLICATION
LN.CNT 2161
       INCLM: 435/287.100
INCL
       INCLS: 604/507.000
              600/573.000; 435/287.100
NCL
       NCLM:
              604/074.000; 604/507.000
       NCLS:
IPC
       [7]
       IPCI
              C12M0001-34 [ICM, 7]; C12M0003-00 [ICS, 7]
       IPCI-2 A61B0005-00 [I,A]; B65D0081-00 [I,A]
              A61B0005-00 [I,A]; A61B0005-00 [I,C]; A61B0010-00 [I,C*];
       IPCR
              A61B0010-00 [I,A]; A61B0010-02 [N,C*]; A61B0010-02 [N,A];
              A61M0001-06 [N,C*]; A61M0001-06 [N,A]; B65D0081-00 [I,C];
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B65D0081-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 35 OF 36 USPATFULL on STN
L9
       2002:32063 USPATFULL
AN
TΙ
       Foam composite
ΙN
       Thomson, Timothy, West Newbury, MA, UNITED STATES
PΙ
       US 20020018884
                           A1 20020214
ΑI
       US 2001-823129
                           A1 20010330 (9)
       Continuation-in-part of Ser. No. US 2000-540099, filed on 31 Mar 2000,
RLI
       PENDING
DT
       Utility
FS
       APPLICATION
LN.CNT 1764
       INCLM: 428/306.600
INCL
       INCLS: 428/319.300
NCL
             428/306.600
       NCLM:
             428/319.300
       NCLS:
IPC
       [7]
       IPCI
              B32B0003-06 [ICM, 7]
       IPCR
              B32B0005-18 [I,C*]; B32B0005-18 [I,A]; C08J0009-00 [I,C*];
              C08J0009-42 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L9
     ANSWER 36 OF 36 USPATFULL on STN
       94:84623 USPATFULL
AN
TΤ
       Apparatus and method for analyzing particles suspended in a liquid
IN
       Spinell, Max, Hilleroed, Denmark
PA
       Biometic ApS c/o Dansk Udviklingsfinansiering A/S, Soeborg, Denmark
       (non-U.S. corporation)
       US 5351118
                               19940927
PΤ
       WO 9117422
                               19911114
       US 1991-688545
ΑI
                               19910610 (7)
       WO 1991-DK120
                               19910506
                               19910610 PCT 371 date
                               19910610 PCT 102(e) date
PRAI
       DK 1990-111990
                               19900504
       Utility
DT
FS
       Granted
LN.CNT 2999
INCL
       INCLM: 356/072.000
       INCLS: 356/335.000; 356/336.000; 356/337.000; 356/338.000; 356/246.000;
              250/283.000; 422/081.000; 422/103.000; 436/805.000; 436/806.000
NCL
       NCLM:
              356/072.000
       NCLS:
              250/283.000; 356/246.000; 356/333.000; 356/335.000; 356/336.000;
              356/337.000; 422/081.000; 422/103.000; 436/805.000; 436/806.000
IPC
       [5]
       IPCI
              G01N0021-01 [ICM, 5]; G01N0021-05 [ICS, 5]; G01N0021-03 [ICS, 5, C*]
       IPCR
              G01N0015-00 [I,C*]; G01N0015-00 [I,A]; G01N0001-38 [N,C*];
              G01N0001-38 [N,A]; G01N0015-10 [I,C*]; G01N0015-10 [I,A];
              G01N0015-12 [I,A]; G01N0015-14 [I,C*]; G01N0015-14 [I,A];
              G01N0021-64 [N,C*]; G01N0021-64 [N,A]; G01N0033-49 [I,C*];
              G01N0033-49 [I,A]
EXF
       356/335; 356/336; 356/337; 356/338; 356/72; 356/73; 356/39; 356/244;
       356/246; 250/283; 422/62.1; 422/81; 422/103; 436/810; 436/805; 436/806;
       324/71.4
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
=> s 19 and peristaltic(p)rollers
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PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH

FIELD CODE - 'AND' OPERATOR ASSUMED 'ERISTALTIC (P) ROLLERS'

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PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'ERISTALTIC (P) ROLLERS'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'ERISTALTIC(P)ROLLERS'
I.10
            12 L9 AND PERISTALTIC (P) ROLLERS
=> d 110 1-12
L10
      ANSWER 1 OF 12 BIOTECHDS COPYRIGHT 2010 THOMSON REUTERS on STN
      2007-13331 BIOTECHDS
AN
TI
      Culturing algae comprises placing algae in aqueous
      medium in a closed system bioreactor, exposing the algae to
      sunlight, and culturing the algae under conditions allowing
      algal reproduction and growth;
         involving cell culture of alga in a photoreactor useful for
         diesel production
      SEARS J T
ΑIJ
      SUNSOURCE IND
PΑ
РΤ
      US 20070048848 1 Mar 2007
      US 2006-510148 24 Aug 2006
ΑI
PRAI
     US 2006-510148 24 Aug 2006; US 2005-711316 25 Aug 2005
DT
      Patent
LA
      English
OS
     WPI: 2007-387522 [36]
L10 ANSWER 2 OF 12 IFIPAT COPYRIGHT 2010 IFI on STN
ΑN
      11398839 IFIPAT; IFIUDB; IFICDB
ΤI
      Closed system bioreactor apparatus
IN
      Sears James T
PΑ
      SUNSOURCE Ind
PΙ
      US 20070048859 A1 20070301
      US 2006-510442
AΙ
                          20060824
                                    (11)
                           20050825 (Provisional)
PRAI US 2005-711316P
      US 2005-733569P
                           20051104 (Provisional)
      US 2005-740855P
                           20051130 (Provisional)
      US 2006-757587P
                           20060110 (Provisional)
      US 2006-818102P
                           20060630 (Provisional)
FΙ
      US 20070048859
                          20070301
      Utility; Patent Application - First Publication
DT
FS
      CHEMICAL
      APPLICATION
      Entered STN: 5 Mar 2007
ED
      Last Updated on STN: 12 Apr 2007
CLMN
     34
    ANSWER 3 OF 12 IFIPAT COPYRIGHT 2010 IFI on STN
T.10
      11398828 IFIPAT; IFIUDB; IFICDB
AN
ΤI
      Method, apparatus and system for biodiesel production from algae
IN
      Sears James T
      SUNSOURCE Ind
PA
PI
      US 20070048848 A1
                          20070301 (CITED IN 001 LATER PATENTS)
ΑI
      US 2006-510148
                          20060824
                                    (11)
     US 2005-711316P
                           20050825 (Provisional)
PRAI
      US 2005-733569P
                           20051104 (Provisional)
      US 2005-740855P
                           20051130 (Provisional)
      US 2006-757587P
                           20060110 (Provisional)
      US 2006-818102P
                           20060630 (Provisional)
FT
      US 20070048848
                          20070301
DT
      Utility; Patent Application - First Publication
FS
      CHEMICAL
      APPLICATION
      Entered STN: 5 Mar 2007
ED
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Last Updated on STN: 12 Apr 2007
CLMN
L10 ANSWER 4 OF 12 USPATFULL on STN
       2009:304349 USPATFULL
ΑN
       DEVICE FOR SPRAYING A COSMETIC COMPOSITION
ΤI
ΙN
       ARNAUD, Pascal, L'Hay Les Roses, FRANCE
       COLLETTE, Annick, St. Maur Des Fosses, FRANCE
       BEAUMARD, Sophie, Villejuif, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
       TIERCE, Pascal, Bondues, FRANCE
PA
       L'Oreal S.A., Paris, FRANCE (non-U.S. corporation)
PΙ
       US 20090272316
                          A1 20091105
ΑI
       US 2009-369854
                           A1 20090212 (12)
       FR 2008-50923
PRAI
                               20080213
       US 2008-71061P
                               20080410 (61)
DT
       Utility
FS
       APPLICATION
LN.CNT 1695
INCL
       INCLM: 118/300.000
NCL
       NCLM:
              118/300.000
IC
       IPCI
              B05B0007-00 [I,A]
       IPCR
              B05B0007-00 [I,C]; B05B0007-00 [I,A]
   ANSWER 5 OF 12 USPATFULL on STN
L10
       2009:230118 USPATFULL
ΑN
ΤI
       DEVICE FOR SPRAYING A COSMETIC COMPOSITION
IN
       ARNAUD, Pascal, L'Hay Les Roses, FRANCE
       BEAUMARD, Sophie, Villejuif, FRANCE
       ROULIER, Veronique, La Varenne St Hilaire, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
       TIERCE, Pascal, Bondues, FRANCE
PA
       L'Oreal S.A., Paris, FRANCE (non-U.S. corporation)
PΙ
       US 20090206174
                          A1 20090820
                           A1 20090212 (12)
ΑI
       US 2009-370217
       FR 2008-50922
                               20080213
PRAI
       US 2008-71060P
                               20080410 (61)
DT
       Utility
       APPLICATION
LN.CNT 1717
INCL
       INCLM: 239/102.200
NCL
       NCLM: 239/102.200
IC
       IPCI
              B05B0001-08 [I,A]; B05B0001-02 [I,C*]
              B05B0001-02 [I,C]; B05B0001-08 [I,A]
       IPCR
L10 ANSWER 6 OF 12 USPATFULL on STN
       2009:153195 USPATFULL
AN
ΤI
       CRANIUM APPARATUS
ΙN
       FALLAH, AFSHIN AL, San Diego, CA, UNITED STATES
PΙ
       US 20090138040
                           Α1
                               20090528
ΑI
       US 2008-325109
                           Α1
                               20081128 (12)
       US 2007-990617P
                               20071128 (60)
PRAI
DT
       Utility
FS
       APPLICATION
LN.CNT 627
TNCL
       INCLM: 606/204.150
NCL
       NCLM: 606/204.150
              A61F0005-00 [I,A]
IC
       IPCI
       IPCR
              A61F0005-00 [I,C]; A61F0005-00 [I,A]
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L10 ANSWER 7 OF 12 USPATFULL on STN
       2007:55917 USPATFULL
ΑN
ΤТ
       Closed system bioreactor apparatus
ΙN
       Sears, James T., Boulder, CO, UNITED STATES
PA
       SUNSOURCE INDUSTRIES (U.S. corporation)
PΙ
       US 20070048859
                           A1 20070301
ΑI
       US 2006-510442
                           A1 20060824 (11)
PRAI
       US 2005-711316P
                                20050825 (60)
       US 2005-733569P
                                20051104 (60)
       US 2005-740855P
                                20051130 (60)
       US 2006-757587P
                                20060110 (60)
       US 2006-818102P
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
    ANSWER 8 OF 12 USPATFULL on STN
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       2007:55906 USPATFULL
ΤI
       Method, apparatus and system for biodiesel production from algae
       Sears, James T., Boulder, CO, UNITED STATES
ΙN
PA
       SUNSOURCE INDUSTRIES (U.S. corporation)
РΤ
       US 20070048848
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       US 2006-510148
                           A1 20060824 (11)
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
    ANSWER 9 OF 12 USPATFULL on STN
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       Combination continuous/batch fermentation processes
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       Pilkington, Phyllis Heather, London, CANADA
       Mensour, Normand Anthony, Ancaster, CANADA
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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       US 20030173272
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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ΑN
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       Apparatus and method for analyzing particles suspended in a liquid
ΙN
       Spinell, Max, Hilleroed, Denmark
PΑ
       Biometic ApS c/o Dansk Udviklingsfinansiering A/S, Soeborg, Denmark
       (non-U.S. corporation)
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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L12
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L12
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      algal reproduction and growth;
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ΑU
      SEARS J T
      SUNSOURCE IND
PΑ
      US 20070048848 1 Mar 2007
PΤ
      US 2006-510148 24 Aug 2006
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      Sears James T
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      SUNSOURCE Ind
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      Entered STN: 5 Mar 2007
      Last Updated on STN: 12 Apr 2007
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L12 ANSWER 3 OF 12 IFIPAT COPYRIGHT 2010 IFI on STN
      11398828 IFIPAT; IFIUDB; IFICDB
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      Sears James T
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      SUNSOURCE Ind
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      Last Updated on STN: 12 Apr 2007
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       ARNAUD, Pascal, L'Hay Les Roses, FRANCE
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       COLLETTE, Annick, St. Maur Des Fosses, FRANCE
       BEAUMARD, Sophie, Villejuif, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
       TIERCE, Pascal, Bondues, FRANCE
       L'Oreal S.A., Paris, FRANCE (non-U.S. corporation)
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L12 ANSWER 5 OF 12 USPATFULL on STN
       2009:230118 USPATFULL
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ΤI
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       ARNAUD, Pascal, L'Hay Les Roses, FRANCE
       BEAUMARD, Sophie, Villejuif, FRANCE
       ROULIER, Veronique, La Varenne St Hilaire, FRANCE
       DURU, Nicolas, Paris, FRANCE
       PRUNIER, Marion, Croissy Sur Seine, FRANCE
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L12 ANSWER 7 OF 12 USPATFULL on STN
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       Closed system bioreactor apparatus
ΤN
       Sears, James T., Boulder, CO, UNITED STATES
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       SUNSOURCE INDUSTRIES (U.S. corporation)
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       US 20070048859
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       US 2006-510442
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L12 ANSWER 8 OF 12 USPATFULL on STN
       2007:55906 USPATFULL
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       Method, apparatus and system for biodiesel production from algae
ΙN
       Sears, James T., Boulder, CO, UNITED STATES
PA
       SUNSOURCE INDUSTRIES (U.S. corporation)
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L12 ANSWER 9 OF 12 USPATFULL on STN
ΑN
       2005:247213 USPATFULL
ΤI
       Combination continuous/batch fermentation processes
       Pilkington, Phyllis Heather, London, CANADA
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                           A1 20050929
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       Continuation-in-part of Ser. No. US 2002-175351, filed on 20 Jun 2002,
RLI
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DT
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L12 ANSWER 10 OF 12 USPATFULL on STN
AN
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ΤI
       METHOD AND APPARATUS FOR MONITORING A FLUID SYSTEM
ΙN
       Staffler, Michael, Furstenfeldbruck, GERMANY, FEDERAL REPUBLIC OF
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L12 ANSWER 11 OF 12 USPATFULL on STN
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TΤ
       Combination continuous/batch fermentation processes
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       NCLS:
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              C12P0007-02 [I,C*]; C12P0007-06 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L12 ANSWER 12 OF 12 USPATFULL on STN
AN
       94:84623 USPATFULL
ΤI
       Apparatus and method for analyzing particles suspended in a liquid
       Spinell, Max, Hilleroed, Denmark
ΙN
PA
       Biometic ApS c/o Dansk Udviklingsfinansiering A/S, Soeborg, Denmark
       (non-U.S. corporation)
PΙ
       US 5351118
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       WO 9117422
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PRAI
                               19900504
DT
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FS
       Granted
LN.CNT 2999
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       INCLM: 356/072.000
       INCLS: 356/335.000; 356/336.000; 356/337.000; 356/338.000; 356/246.000;
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       356/246; 250/283; 422/62.1; 422/81; 422/103; 436/810; 436/805; 436/806;
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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L12
      ANSWER 1 OF 12 BIOTECHDS COPYRIGHT 2010 THOMSON REUTERS on STN
ΤI
      Culturing algae comprises placing algae in aqueous
      medium in a closed system bioreactor, exposing the algae to
      sunlight, and culturing the algae under conditions allowing
      algal reproduction and growth;
         involving cell culture of alga in a photoreactor useful for
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diesel production
DERWENT ABSTRACT:

AB

NOVELTY - Culturing algae comprises: (a) placing algae in aqueous medium in a closed system bioreactor comprising one or more flexible tubes operably coupled to one or more peristaltic rollers; (b) exposing the algae to sunlight; (c) culturing the algae under conditions allowing algal reproduction and growth; and (d) using the rollers to move the medium through the tubes, where the movement of the rollers removes photosynthetic oxygen from the tubes and scrubs the surface of the tubes to reduce biofilm on the tube surfaces.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a system for producing biodiesel from algae comprising: (a) a closed bioreactor comprising two flexible tubes operably coupled to two peristaltic rollers, the tubes containing a suspension of algae in aqueous medium; (b) a mechanism for harvesting the algae from the medium; (c) a device for separating oil from the algae; and (d) an apparatus for converting the oil into biodiesel.

BIOTECHNOLOGY - Preferred Method: In culturing algae, the bioreactor comprises 2 tubes, each tube operably coupled to a different roller. The tubes comprise a thermal barrier. The method further comprises diverting medium above or below the thermal barrier to regulate the temperature of the medium. It also comprises separating algae from the medium, by removing oil from the algae. The method also comprises producing biodiesel from the oil, where the biodiesel is produced by transesterification. The method further comprises. axial vortex inducers to induce formation of rotating water columns within the tubes, the rotation of the water columns moving algae between the light-exposed upper region and the darker lower regions of the tube. The adjacent water columns in the tube. . . clockwise or counterclockwise directions. The method further comprises introducing CO2 gas into the medium using one or more CO2 bubblers. Algae are partially separated from the medium using a whirlpool device. It further comprises separating non-oil products from the algae, where the non-oil products comprise carbohydrates. The carbohydrates are converted into hydrogen gas, methane gas, and/or ethanol. The method further comprises harvesting the algae for use in animal or human food. Preferably, the algae are Spirulina, Dunaliella, or Tetraselmis. It further comprises using the algae as food for an algae-eating aquatic species, where the aquatic species is a peneid shrimp. In the method above, the tubes are arranged in a. to cool the suspension and below the thermal barrier to maintain the temperature of the suspension at ground temperature. The rollers reverse direction when they reach the ends of the tubes. Dip and belly pans are located below the roller at each end of each tube to allow medium to flow under the roller. The method further comprises controlling the movement of the rollers to prevent skewing. Preferred System: In the system above, the rollers are arranged to roll down the length of the flexible tubes to move the suspension through the tubes. The mechanism for harvesting algae comprises a whirlpool device and one or more sipper tubes. The whirlpool device comprises a speed-up ramp, a dwell tube, and a slowdown ramp. The rollers reverse direction at the ends of the tubes, where the positions of the speed-up and slowdown ramps are reversed when the rollers reverse direction. The mechanism for harvesting algae comprises at least one centrifuge. The apparatus for converting oil into biodiesel utilizes a transesterification process. In the system above, the rollers in contact with the tubes are arranged so that they compress the tubes to 85% of the height of the. formation of a fluid vortex within the whirlpool device. The whirlpool fluid movement results in a partial separation of oil-containing

algae from the aqueous medium. The tubes contain a thermal barrier arranged horizontally within the tubes, to regulate the temperature of. . . ceramic or plastic, a silicate, or glass. It also exhibits an infrared emissivity of close to 1.0. Movement of the rollers along the tubes collects oxygen and other gases from the medium for removal from the system. The upper layer of. . . indented with a linear Frenel pattern that collects sunlight from a lower Snell's law angle and directs it into the algae growing medium. The tubes are laid out perpendicular to the low angle southern sun for the winter months in temperate climates.

USE - The method and system are useful for culturing algae and for producing useful products from algae, including biofuels (e.g. biodiesel, methanol, ethanol), bio-polymers, chemical precursors, and/or animal or human food. The algal culture can be used to provide animal or human food source, by culturing edible algae. It can also be used to support growth of a secondary food source, such as shrimp or other aquatic species that feed on algae. (46 pages)

CT ALGA, CELL CULTURE, PHOTOREACTOR, CULTURE MEDIUM, APPL., DIESEL PREP., METHANOL, ETHANOL, BIOPOLYMER, CHEMICAL PRECURSOR, FOOD-ADDITIVE, FEEDSTUFF CULTURE ALCOHOL BIOREACTOR (26, 26)

=> d 112 12 ab

L12 ANSWER 12 OF 12 USPATFULL on STN

An apparatus and a method for analyzing particles suspended in a liquid are described, which particles are of a type or are prepared so as to emit radiation characteristic of the particles when exposed to radiation. Particular fluorescence-photometrical flow cytometry is described in connection with biological cell populations.

The apparatus comprises an elongated flow area in the shape of a channel provided in a cuvette comprising a first body member having a planar facial surface and a second body member having a planar facial surface. The first and the second body members are adapted to be moved relative to one another between a first position, in which the planar facial surfaces are in fluid-tight contact with one another, and a second position, in which the planar facial surfaces are spaced apart. At least one of the planar facial surfaces of one of the body members is provided with an elongated groove. In the first position, the first and second body members together define the cuvette in which the elongated flow area is defined by the elongated groove constituting a capillary passage. An inlet for feeding the liquid to the elongated flow area is provided in one of the body members and an outlet for discharging the liquid from the elongated flow area is provided in the very same or in the other body member. At least one of the body members is transparent to light to which the particles are exposed and further transparent to the radiation emitted by the particles. Two electrodes are further described for carrying out an election of particles suspended in the liquid by means of a measurement of the electrical conductivity of the fluid, which particles are of a specific size for subsequent registration of fluorescence when exposed to radiation.

=> d 112 12 kwic

L12 ANSWER 12 OF 12 USPATFULL on STN

SUMM The invention relates particularly to analyzing biological cells by means of flow cytometry, more particularly to flow cytometrically analyzing biological cells which are labelled with a fluorescent antibody or which are anti-fluorescent.

- SUMM Several cells, both circulating and "fixed" cells, have on their superficial structure so-called antigenic determinants which are specific to the cells in question. Moreover, a number of antigenic determinants are common to different cells, which antigenic determinants may be used as the basis for a general classification.
- SUMM . . . monoclonal antibodies is very rapid and today there is access to monoclonal antibodies that react with e.g. most human blood cells and their respective subgroups. Furthermore, antibodies capable of reacting specifically with different cancer cells have been cloned.
- Antibodies of both of the above-mentioned types may be used for fluorescent-microscopically analyzing cells after conjugation with a Suitable fluorochrome, particularly of the type having green and red fluorescence, e.g. FITC (fluorescein isothiocyanate) and. . . suitable filter combinations in fluorescence microscopes for visualizing the bond of two different antibodies ("green" and "red", respectively) to biological cells simultaneously. Other fluorescent tests may be applied for visualizing and quantifiying cellular components. Thus, ribonucleic acids are detectable by the. . . analogues, the cleavage of which results in the formation of a fluorescent product (fluorescein diacetate, methyl umbelly furones). Furthermore, several cells posses specific receptors capable of binding ligands. These ligands are often involved in intercellular recognition and control operations. Fluorescence-labelled ligands (or ligand analogues) may be used for detecting receptors of this type. Finally, several bacteria and algae posses auto-fluorescent properties.
- SUMM The use of fluorescence spectrophotometry is particularly relevant in connection with the examination of subgroups of the type of cells which can only be distinguished from one another by means of superficial markers which are visualized by means of antibodies....

 the basis of morphological criterions, but on the basis of functional and antigenic differences. Subsequent to antigenic stimulation, the B cells can produce antibodies. These antibodies are exposed on the surface of the B cells and can be utilized for classification by the use of anti-antibodies. The T cells fulfil a number of different functions, both in the control of the immune apparatus and in the direct execution of the cellular immunity. Similarly, the T cells can be divided into a number of subgroups by monoclonal antibodies. Consequently, the examination of the distribution on the various. . .
- SUMM . . . to block. Moreover, the analysis normally comprises all fluorescent particles, whether they have a size corresponding to that of the cells to be analyzed or not. Apparatuses of the type mentioned above have been disclosed in the patent literature, cf. e.g..
- SUMM The present invention provides an apparatus and a method suitable for analyzing fluorescence-labelled or autofluorescent particles of cells suspended in a liquid, which apparatus, however, does not suffer from the disadvantages of the above-mentioned prior art apparatuses, which. . .
- SUMM The invention is to be explained in greater details below, particularly relating to flow-cytometrically analyzing biological cells which are flourescent having a characteristic fluorescent radiation when subjected to illumination, it is, however, to be understood that the. .
- SUMM Moreover, the invention will be explained particularly in relation to a fluid medium in which biological cells to be analyzed are suspended, which fluid medium is passed through a passage having cross-sectional dimensions adapted to the size of the cells, and which fluid medium is exposed to light for illuminating the cells individually. Consequently, the existence of an elongated

flow area having the dimensions of a capillary passage is mandatory, however, causing. . .

- SUMM . . . of the apparatus and the method according to the present invention, which embodiments particularly tel ate to spectrophotometrically fluorescence-analyzing biological cells , a unique technique is provided, which is well-suited for the aforesaid analyzing of particular cell populations comprising subgroups of cell.
- SUMM . . . mm, preferably not exceeding 100 μm , and most preferably 30-35 μm . This dimension of the passage is suitable for biological cells having a diameter of the order of 8-15 μm , which is the normal size of some lymphocytes. The above-mentioned dimensions. .
- SUMM . . . for agitating particles suspended in a liquid. Surprisingly this wire body has proved capable of avoiding any agglomeration of biological cells, capable of avoiding that the particles stick to the the surface of the passage and capable of ensuring that the.
- DETD . . . which causes a rotor 44 to rotate relative to a rotational axis 45. The rotor 44 is provided with pressure rollers 46, the axes of which extend radially from the rotor. A stationary component of the hose pump 41 is shown. . .
- DETD The pressure rollers 46 influences the tube axially so as to flatten the internal aperture of the tube completely at a section below each roller, so as to produce a peristaltic pumping action when the rotor 44 rotates. Due to the tensile stress of the tube, the tube is influenced by. . . the groove when inflated. In the embodiment shown in FIG. 7, the hose pump comprises a total of four pressure rollers, which are arranged at an angular spacing of 90°.
- DETD This hose pump ensures a continous and well-controlled pumping, however, a discontinuity cannot be totally avoided whenever a roller rolls accross the section of the discharge holes 47, and consequently the tube bends. When a pressure roller leaves one end of the tube and hits the opposite end, a step-wise variation of the tube pressure is unavoidable.. . . be completely steady, this may be achieved by measuring only during the time intervals between the passage of the pressure rollers across the tube bends, i.e. in four periods per turn of the rotor 44 shown in FIG. 7. However, these. . reference numeral 35 in its entity, differs from the hose pump shown in FIG. 7 in that only two pressure rollers are provided having axes arranged 60°-90° spaced apart. Hereby a suitable time of measuring is achieved with a completely continuous. . . one rotation of the rotor 44 at approximately 240°-270°, viz. 270°-300° minus the period, which is required for the pressure roller to move across the tube bends at the discharge holes 47.
- DETD . . . cuvette 3, 4 is open for a short period of time of the order of 20 ms as the lens roller 61 supporting the plate 4 is moved away from the plate 3, as explained above with reference to FIGS. 3. .
- DETD . . . radiation, emit radiation characteristic of the particles. In the preferred embodiments, the apparatus is particularly suited for analyzing small, biological cells, e.g. lymphocytes of a magnitude of the order of 8-15 μm , by means of fluorescence spectrometry. The commercially available, prior. . .
- DETD . . . a flow microscope, in which relevant particles are illuminated and visually registrated, or as an image storage system, in which cells are photographed for the purpose of subsequent, morphologically image analyzing. Also focusing of the particles is used by forcing the. . .

(FILE 'HOME' ENTERED AT 19:26:08 ON 09 NOV 2010)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 19:26:16 ON 09 NOV 2010 SEA PERISTALTIC(P)ROL? AND CULTUR?(P)ALGA?

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                  FILE WPINDEX
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                QUE PERISTALTIC (P) ROL? AND CULTUR? (P) ALGA?
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              9 S L1
L3
              6 DUP REM L2 (3 DUPLICATES REMOVED)
L4
              4 S L3 AND ROLLER?
L5
           5331 S PERIST? AND ROLLER?
           2039 S L5 AND (ALGA OR ALGAE OR MICROORGANISM OR ORGANISM OR BACTER
L7
           1840 S L6 AND PERISTALTIC
L8
            896 S L7 AND ROLLERS
L9
             36 S L8 AND ALGAE
L10
             12 S L9 AND PERISTALTIC (P) ROLLERS
1.11
             12 S L10 AND ALGA?
             12 S L11 AND ALGAE
L12
=> s 112 and bioreactor
             7 L12 AND BIOREACTOR
L13
=> d 113 1-7
L13
      ANSWER 1 OF 7 BIOTECHDS COPYRIGHT 2010 THOMSON REUTERS on STN
      2007-13331 BIOTECHDS
ΑN
      Culturing algae comprises placing algae in aqueous
TΤ
      medium in a closed system bioreactor, exposing the
      algae to sunlight, and culturing the algae under
      conditions allowing algal reproduction and growth;
         involving cell culture of alga in a photoreactor useful for
         diesel production
ΔII
      SEARS J T
PΑ
      SUNSOURCE IND
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US 20070048848 1 Mar 2007
PΤ
ΑТ
      US 2006-510148 24 Aug 2006
PRAI
     US 2006-510148 24 Aug 2006; US 2005-711316 25 Aug 2005
DT
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LA
      English
      WPI: 2007-387522 [36]
OS
L13
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      11398839 IFIPAT; IFIUDB; IFICDB
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      Closed system bioreactor apparatus
ΙN
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PA
      SUNSOURCE Ind
PΙ
      US 20070048859 A1
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      US 2006-510442
                          20060824
                                    (11)
     US 2005-711316P
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                           20060630 (Provisional)
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FS
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      APPLICATION
      Entered STN: 5 Mar 2007
      Last Updated on STN: 12 Apr 2007
CLMN
L13
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AN
      11398828 IFIPAT; IFIUDB; IFICDB
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ΤN
      Sears James T
PA
      SUNSOURCE Ind
PΤ
      US 20070048848 A1 20070301 (CITED IN 001 LATER PATENTS)
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      Utility; Patent Application - First Publication
FS
      CHEMICAL
      APPLICATION
ED
      Entered STN: 5 Mar 2007
      Last Updated on STN: 12 Apr 2007
CLMN
     47
L13 ANSWER 4 OF 7 USPATFULL on STN
       2007:55917 USPATFULL
AN
ΤI
       Closed system bioreactor apparatus
ΙN
       Sears, James T., Boulder, CO, UNITED STATES
PA
       SUNSOURCE INDUSTRIES (U.S. corporation)
PΙ
       US 20070048859
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       US 2006-510442
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                               20060824 (11)
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       US 2006-818102P
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FS
       APPLICATION
LN.CNT 1648
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       IPCR
              C12M0003-00 [I,C]; C12M0003-00 [I,A]
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       2007:55906 USPATFULL
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       Sears, James T., Boulder, CO, UNITED STATES
ΙN
       SUNSOURCE INDUSTRIES (U.S. corporation)
PA
PΤ
       US 20070048848
                            A1 20070301
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PRAI
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                                20050825 (60)
       US 2005-733569P
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       INCLS: 435/257.100; 554/174.000
              435/134.000
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       NCLS:
              435/257.100; 554/174.000
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              C07C0051-42 [I,C*]
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              C07C0051-43 [I,A]; C12N0001-12 [I,C]; C12N0001-12 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L13 ANSWER 6 OF 7 USPATFULL on STN
ΑN
       2005:247213 USPATFULL
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       Combination continuous/batch fermentation processes
ΙN
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       Mensour, Normand Anthony, Ancaster, CANADA
PΙ
       US 20050214408
                            A1 20050929
       US 2005-98688
                                20050405 (11)
AΙ
                            A1
       Continuation-in-part of Ser. No. US 2002-175351, filed on 20 Jun 2002,
RLI
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DT
       Utility
FS
       APPLICATION
LN.CNT 5429
INCL
       INCLM: 426/016.000
       INCLS: 435/161.000
NCL
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       NCLM:
              435/161.000
       NCLS:
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
    ANSWER 7 OF 7 USPATFULL on STN
T<sub>1</sub>13
       2003:219769 USPATFULL
AN
ΤI
       Combination continuous/batch fermentation processes
IN
       Pilkington, Phyllis Heather, London, CANADA
       Mensour, Normand Anthony, London, CANADA
PΙ
       US 20030153059
                            A1 20030814
ΑТ
       US 2002-175351
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       US 2001-299186P
                                20010620 (60)
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       APPLICATION
LN.CNT 6316
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INCL
       INCLS: 426/016.000
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              C12P0007-02 [I,C*]; C12P0007-06 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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=> d 113 7 kwic

L13 ANSWER 7 OF 7 USPATFULL on STN

- AB . . . pitch and/or initially ferment a wort containing fermentable sugars. An exemplary continuous stage involves the use of a gas lift bioreactor employing superflocculant yeast and stringent oxygen control. The continuous stage can then be followed by sending an at least partially. . .
- SUMM . . . the United States ran some preliminary evaluations on a Meura Delta test unit, as well as on the fluidized bed bioreactor distributed by Schott Engineering. Coors Brewing also performed preliminary experimentation with the Meura Delta system.
- SUMM . . . Domeny, 1999). Guinness initially investigated the use of various adsorption carriers for immobilization and subsequent fermentation in a fluidized bed bioreactor (Donnelly, 1998).

 In 1999, Donnelly and colleagues published a paper describing the kinetic of sugar metabolism inside their fluidized bed bioreactor (Donnely et al., 1999). Their experimental setup involved the use of Siran porous glass beads as the immobilization carrier for. . .
- SUMM [0005] A team from the Sapporo Breweries Ltd. Brewing Research Laboratories located in Japan studied the use of immobilized cells in a fluidized bed reactor for the main fermentation of beer. Their studies involved the use of polyvinyl alcohol gel. . and chitosan gel beads (Shindo et al., 1994c) as immobilization matrices. In the latter study, a one liter working volume bioreactor containing 25% by volume Chitopcarl® type II beads (chicosan beads) was operated on a continuous basis with wort treated with. . .
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- SUMM . . . particular there is provided a preferred process in which the continuous fermentation is carried out using a gas lift type bioreactor, employing a flocculent (and especially a highly flocculent or superflocculant) yeast strain and employing stringent oxygen control.
- SUMM [0406] The process according to claim 1 wherein the continuous stage is carried out in a gas lift bioreactor In accordance with a continuous stage useful in the various practices under the present invention, it is preferred that immobilized cells be utilized, (as opposed to purely free cells) and this may be carried out using a selected one of carrier immobilized or flocculating yeasts. Notwithstanding the forgoing, it is preferred that flocculating yeasts be used instead of carrier immobilized cells, and superflocculant yeasts are especially preferred for this purpose.
- DETD [0425] Two types of adsorption matrices were tested in the pilot scale

gas-lift draft tube bioreactor. Pictures of both these carriers are presented herein. Schott Engineering provided a sintered glass bead carrier, Siran®. The selected particles. . . immobilization with a 55-60% pore volume and pore size distribution between 60 and 300 .quadrature.m, an appropriate size for yeast cells. This type of carrier is reported to be biologically and chemically stable, easy to clean, reusable, sterilizable with stearn, non-compacting. . .

- DETD [0434] Liquid samples containing freely suspended yeast cells are first collected from the fermentation medium by the above sampling procedure. A Hauser Scientific Company Hemacytometer with a volume. . . liquid samples should be diluted with distilled water in order to achieve a total yeast count of 150 to 200 cells in the counting field. Heggart et al. (1999) describe all the factors that affect viability and vitality characteristics of yeast. . . described by the American Society of Brewing Chemists was used (Technical Committee and Editorial Committee of the ASBC, 1992). Live cells can render the metylene blue stain colorless by oxidizing it. Dead cells, on the other hand, will stain blue. The following reagents were used in the preparation of methylene blue for viability. . .
- DETD . . . in a test tube and then thoroughly mixed. After allowing this mixture to rest for several minutes (ensures contact between cells and the dye), a drop of liquid was placed between the hemacytometer's counting glass and the cover slip (defined volume). The percentage of viable cells was determined by counting both the viable and dead cells within the counting field and then dividing the number of viable cells by the total number of cells.
- DETD [0441] When using yeast cells with a tendency to form flocs, it becomes difficult to accurately assess the number of cells present in a liquid sample because the cells will tend to settle in the sample jar. In order to obtain a representative sample, a deflocculating agent was used. In these experiments, a 0.5% by volume sulfuric acid solution was employed to destabilize the flocculated yeast cells, hence allowing for a representative yeast cell count. The same enumeration and viability procedure outlined in section 4.5.1 was used. . .
- DETD [0443] Before yeast counts were performed on gel-entrapped cells , it was necessary to disrupt the gel matrix using a Polytron® apparatus (Brinkmann Instruments). A sample of beads was first.
- DETD [0472] In order to measure mixing time and circulation rate inside the three phase gas-lift draft tube bioreactor, an acid injection system linked to a data acquisition system was utilized. By applying a pulse of a strong acid into the bioreactor, it was possible to calculate both circulation rate and mixing time by monitoring the change in pH over time and. . .
- DETD [0477] Ten milliliters of 10 N hydrochloric acid were injected into the annulus section of the gas-lift draft tube bioreactor (diagram is provided in FIG. 5.5) just under the pH probe location. This distance corresponded to a height of 26. . .
- DETD [0479] These mixing experiments were conducted on actual fermentations inside the 50-L gas-lift bioreactor with one of three immobilization carriers present (either superflocculent yeast LCC290, medium flocculent yeast LCC3021 or .quadrature.-carrageenan gel beads) The. . .
- DETD [0480] Design of a Pilot Scale Gas-lift Draft Tube Bioreactor System Gas-Lift Draft Tube Bioreactor Fermentation System
- DETD . . . was set up to feed up to 3 independent fermenters through a valve header system (V7, V8 & V9). Masterflex peristaltic pumps (P1& P2) were utilized to deliver a prescribed flow of wort to the pilot scale bioreactors (R1 & R2).. . . air inlet

```
NV4
             Needle valve at carbon dioxide inlet
NV5
             Needle valve at air inlet
NV6
             Needle valve at carbon dioxide inlet
Р1
             Masterflex peristaltic feed pump for R1
P2
             Masterflex peristaltic feed pump for R2
PR1
             Carbon dioxide pressure regulator
PR2
             Carbon dioxide pressure regulator
PR3
             Air pressure regulator
PR4
             Carbon dioxide pressure regulator
PR5
             Air pressure regulator
PR6
             Carbon dioxide pressure regulator
              Pilot scale gas-lift draft tube bioreactor;
R1
              50 L working volume
R2
              Pilot scale gas-lift draft tube bioreactor;
              50 L working volume
              Carbon dioxide rotameter; 0 to 20 scfh scale
RM1
              Carbon dioxide rotameter; 0 to 20 scfh scale
RM2
RM3.
DETD
       [0484] More detailed diagrams and exact dimensions of the 50-L pilot
       scale bioreactor are provided in section 5.1.1. The wort
       handling and storage protocol will be presented in section 5.1.2 while
       the cleaning.
DETD
       [0486] The 50-L working volume bioreactor designed for this
       work was built entirely of 304L stainless steel with 4 Plexiglas look
       windows located in the body.
       . . with dimensions provided in centimeters. FIGS. 5.4 to 5.6 are
DETD
       detailed sectional drawings of the 50 L gas-lift draft tube
       bioreactor and FIG. 5.7 is a schematic of the gas sparging
       device utilized in these experiments.
DETD
       [0501] The wort feed line was connected to the bioreactor
       after an internal temperature of 20° C. was reached. With valves
       V2, V5, V10 and V14 still in the closed.
       . . . passing this gas mixture through the sterile filter (Millipore,
DETD
       Millex®-FG.sub.50, 0.2 .quadrature.m Filter Unit) and into the
       draft-tube of the bioreactor. A superficial air velocity of
       0.39 \text{ mm/s} (0.4 \text{ scfh}) was injected into the reactor for all the
       fermentations, while the primary mixing gas flowrate was adjusted to
       suit the specific immobilization type. The 50-L gas-lift
       bioreactor followed a traditional batch start-up before a
       continuous mode of operation was started. After cleaning and
       sterilization as described in section 5.1.2, the gas-lift
       bioreactor was filled with 50 liters of wort from the wort
       holding tanks (WT1 or WF2) and then injected with 200. .
DETD
       . . . analyses (methods described in Chapter 4). At selected time
       periods, continuous fermentation product was collected from the 50-L
       primary fermentation bioreactor in larger quantities (40-L
       sterile stainless steel cans) and subjected to post-fermentation
       processing in order to produce a finished, saleable beer for evaluation
       and comparison to industrially-produced control beer. The selected 50-L
       bioreactor was disconnected from the waste beer vessel and
       immediately connected to the beer collection vessel. Once the desired
       liquid had been collected, the bioreactor was reconnected to
       the waste beer vessel. The collected "green" beer was subjected to a
       post-fermentation hold period in order. . . liquid's diacetyl level
       below 30 .quadrature.g/L. The yeast carried over with the liquid was
       allowed to settle and the liquid (cells concentration of
       .about.1-5 million cells/mL) was placed in cold storage for
       aging (7 days at 2^{\circ} C.). After the aging period, the liquid was
       filtered,. .
       . . . evaluate a continuous bead production process for the
DETD
       production of yeast-inoculated gel beads in order to supply immobilized
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LCC3021 yeast cells to the 50-L continuous gas-lift draft tube

bioreactors described in section 5.1.

- DETD . . . an emulsion between the non-aqueous continuous phase (vegetable oil) and the aqueous dispersed phase (K-carrageenan gel solution mixed with yeast cells) with the use of static mixers. Rapid cooling to induce polymer gelation followed this step. The formed beads were then. . .
- DETD . . . in a temperature regulated water bath and the yeast inoculum was maintained at 20° C. prior to immobilization. Using Masterflex peristaltic pumps (Cole Parmer Company, USA), the gel and the yeast slurry were pumped through 24 elements of the 6.4 mm diameter static mixer in order to disperse the cells evenly through the gel. The sterilized oil, stored at room temperature, was pumped (Masterflex peristaltic pump) into the hot water bath to also reach a temperature of 37° C.
- DETD . . . beads were the oil and polymer. κ-Carrageenan (type X-0909, lot 330360, Copenhagen Pectin, Denmark), a polysaccharide polymer extracted from red algae, was a generous gift from Copenhagen Pectin A/S. This polymer possesses the unique property of thermo-gelation, where its gelling temperature. . .
- DETD [0522] The use of immobilized cells for the production of ethanol has been published. In the last two decades, researchers have attempted to optimize the ethanol. . .
- DETD [0524] Batch fermentations utilizing freely suspended yeast cells were conducted in the 50-L pilot scale gas-lift draft tube bioreactor. These trials provided the opportunity to assess the feasibility of using such a system for the fermentation of wort into. . . served to establish a benchmark for future comparison with continuous fermentation liquids. Two batch fermentations were undertaken in the 50-L bioreactor using a lager yeast strain from the Labatt Culture Collection (LCC3021). The yeast growth rate, as well as the consumption. . .
- DETD . . . slowly at the beginning of the fermentation due to the presence of oxygen in the fermentation medium as the yeast cells are in their aerobic growth phase. Once the oxygen has been depleted, ethanol levels rise exponentially until the fermentable sugars. . .
- DETD . . . the results presented in this section, it appeared that the two batch fermentations proceeded normally within the gas-lift draft tube bioreactor. Yeast growth and carbohydrate uptake followed the expected paths, as did the by-products, ethanol, diacetyl and pentanedione. A comparison of. . . 100 hours as compared to 120-168 hours for traditional batch lager fermentation. The agitation provided by the gaslift draft tube bioreactor contributed to this decrease in fermentation time due to the enhanced mass transfer afforded by such systems. With this information, future fermentation trials were performed with confidence that the gas-lift draft tube bioreactor did not significantly alter the fermentation metabolism of yeast and that fermenting with freely suspended yeast within this system could. .
- DETD . . . most promising alternatives for future development work. Three distinct modes of immobilization were tested within the 50-L gas-lift draft tube bioreactor. Two commercially available adsorption carriers with sizes ranging between 1 and 2 mm were evaluated. Siran®, a glass bead carrier. . .
- DETD [0530] The initial fermentation results with both of these carriers in the 50-L gas-lift draft tube bioreactor were unfavorable. The problems that arose were mainly due to the high particle densities of Sirar® and Celite® as compared. . . liquid phase was a significant increase in the minimum gas fluidization velocity required to operate the 50L gas-lift draft tube bioreactor. For 4 liters of Siran® carrier (8% v/v solids loading), a gas velocity of 21.5 mm/s (based on draft tube. . .
- DETD [0533] Entrapment-based immobilization methods require the inclusion of

the yeast cells within the matrix prior to their introduction into the fermentation vessel. Since in-situ reactor inoculation is not feasible at this. . .

- DETD . . . be considered. An increase in volumetric productivity of the system is necessary in order to supply the volume of immobilized cells required to feed a large-scale bioreactor. For example, a 2000-hL gas-lift draft tube bioreactor would require approximately 800 hL of beads. To achieve such volumes, an increase in both the flows of gel and. . .
- DETD . . . high production temperature, both heating and cooling systems are required in the process. The potential thermal shock that the yeast cells are exposed to requires additional investigation so that an assessment can be made as to potential negative implications. In this. . .
- DETD . . . oil and the method of removing this saline solution from the bead slurry before the introduction of beads into the bioreactor requires attention. Otherwise it may be accessary to flush this solution from the reactor following the addition of beads to. . .
- DETD [0554] Since the immobilized cell beads are produced outside the bioreactor, aseptic techniques must be utilized throughout the bead formation process and sterility maintained until the beads are introduced into the bioreactor. The various transfer points between tanks provide opportunity for contamination and must be monitored due to the fact that the. . .
- DETD [0555] 6.2.2 Flocculent Yeast Cells
- DETD [0556] One of the most natural form of immobilization is the self-aggregation of microorganism into flocs of cells
 . Calleja and Johnson (1977) have proposed three possible reasons for cells to come in contact with each other to form aggregates, with all distinctive bonding properties. The first involves cells of different sexes being attracted to each other by the release of pheromones (.quadrature. and a-factors). This type of bonding. . .
- DETD [0557] Cells may also aggregate through their failure to separate from the mother cell during the budding process. This failure may be. . . mutation of a number of genes. This phenomenon is referred to as chain-formation and not flocculation. The bonds between these cells can be irreversibly destroyed by mechanical shear (Stratford, 1996). The third scenario is more commonly known as flocculation. Stewart and Russell (1981) have defined flocculation as a reversible "phenomenon wherein yeast cells adhere in clumps and either sediment rapidly from the medium in which they are suspended or rise to the medium's. . . between adjacent cell walls. More specifically, it is thought that specific lectins are bound to the .quadrature.-mannans of the adjoining cells in the presence of Ca.sup.2+ions (Calleja and Johnson, 1977). This protein/carbohydrate bonding was found to be reversibly inhibited by chelating. .
- DETD . . . yeast cell configurations, namely non-flocculent yeast, chain-forming yeast and flocculent yeast. In the case of the chain-forming yeast, although the cells have aggregated, it is not considered as a type of flocculation since these cells were never single to start with and flocculation implies single cells coming together to form a mass because of favorable environmental conditions (Ca.sup.2+ ions and low levels of inhibiting sugars). In the case of flocculent cells, the specific size of the floc may be dependent on cell genetics as well as on the hydrodynamic conditions to. . .
- DETD [0565] Before performing continuous fermentations with the superflocculent yeast LCC290 within the gas-lift draft tube bioreactor, it was decided to characterize the yeast in lab scale, shake flask fermentations. FIG. 6.28 shows the evolution of the.

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- DETD [0566] FIG. 6.30 shows the consumption of carbohydrates over the course of fermentation. The yeast cells first consumed the simple sugars glucose and fructose then sequentially took up maltose and malrotriose. Brewing yeast cannot, however, metabolize. . .
- DETD . . . hence flocculation will commence only once this inhibitor has been depleted. In the sample at 40 hours batch fermentation, the cells began to flocculate and settled out of solution when tested using the method described in section 4.7. Settling was very. .
- DETD . . . continuous fermentations to indicate where the pseudo-steady-state liquid specific gravity should be kept if it is desired to keep the cells flocculated. Operating the reactor above 6° P would risk destabilizing the flocculated cells and possibly lead to the washout of the immobilized yeast population. The settling characteristics of the superflocculent yeast indicate that the yeast population will settle out quite quickly if left stagnant. With a three-phase gas-lift draft tube bioreactor, it will be possible to keep these cells in circulation, however, in the case of a failure in the gas supply system, the cell population would settle out. . .
- DETD . . first and most important goal of this thesis was to evaluate the feasibility of operating the 50-L pilot scale gas-lift bioreactor in continuous mode using .quadrature.-carrageenan gel beads to entrap Saccharomyces carlsbergensis cells (described in section 6.2.1). In addition, it was our desire to investigate whether a North American type lager beer of. . . fermentation time of five to seven days. The dissolved oxygen concentration measured by the in-place Ingold oxygen probe within the bioreactor was close to zero regardless of the oxygen added to the sparging gas (ranged from 0 to 20% v/v). This indicated that the oxygen supplied in the wort was either consumed quickly by the yeast cells or was simply vented in the off-gas. The level of free cells in the overflow of beer was in the order of 10.sup.8 cells per mL of green beer. The levels of the vicinal diketones, diacetyl and 2,3-pentanedione, as well as the level of.
- DETD . . . was judged unacceptable with signs of flavor oxidation and a "papery" and "winey" taste. At pseudo-steady state, the pilot scale bioreactor was operated with a residence time of 24 hours over a 6-week period. The "green" beer had an acceptable flavor. . .
- DETD . . . before being transferred into the fermenter. After inoculation of the medium, the dissolved oxygen concentration rapidly decreases as the yeast cells consume it (first 24 hours of fermentation where yeast growth occurs). The remainder of the fermentation is therefore carried out. . .
- DETD [0575] Regardless of these differences, the bioreactor configuration tested in this initial assessment produced a beer with an acceptable flavor quality and analytical profile. By using a gas-lift bioreactor with relatively small-sized beads (.about.1 mm), it was possible to increase the volumetric bioreactor productivity by reducing the time for primary fermentation by several days. The level of biomass released in the exiting beer showed that the level of yeast growth in the immobilized cell bioreactor was equivalent to that of free cell batch fermentation under similar conditions. These observations confirm the reliance of flavor formation.
- DETD . . . performed on three types of immobilized cell fermentations. The experimentation was carried out inside the 50-L pilot scale draft tube bioreactor on a model water solution containing no solids and then on fermentation broth with a specific gravity of 2.7° P. .
- DETD . . . for the LCC3021 and the .quadrature.-carrageenan system were between the water/no solids and the LCC290 systems. Solids within the

- gas-lift bioreactor help with the dispersion of liquid phase fluid elements by stimulating the formation of eddies and promoting co-axial mixing. The. . .
- DETD [0584] FIG. 6.49 Mixing Time versus Superficial Gas Velocity in a 50 L Gas-lift Draft Tube Bioreactor. An Acid Pulse was Injected into a Fermentation Medium Containing the Highly Flocculent Yeast, LCC290 (Floc Size>1.0 mm). Mixing Time. . .
- DETD . . . difference in mixing time versus residence time strongly suggest an adequately mixed system. The original premise for using a gas-lift bioreactor was that it provided an ideally mixed environment for beer fermentation. The results of the mixing studies performed on all. . .
- DETD [0589] Continuous fermentations were performed in the 50-L pilot scale gas-lift draft tube bioreactor utilizing three types of immobilization carrier—.quadrature.—carrageenan gel beads, superflocculent LCC290 yeast and medium flocculent LCC3021 yeast. All fermentations were initially. . . the flocculent yeast, this batch phase allowed for the formation of yeast flocs, which could then be retained within the bioreactor once a continuous feed was started. Once the diacetyl level in the fermentation liquid had dropped to below 30 .quadrature.q/L, . .
- DETD . . . continuous wort feed. In batch fermentation, it is common for viability to decrease at the end of fermentation since the cells are deprived of nutrients. Once the continuous wort feed was started, viability climbed back above 90%. The free yeast cell. . . the first 400 hours of fermentation and then, over the next 300 hours, it increased about tenfold from .about.100 million cells per mL to .about.1.5 billion cells per mL. Once at this maximum concentration, the free cell yeast population maintained this pseudo-steady state value for the remainder. . .
- DETD . . . at .about.700 hours, the yeast had no more room to grow within the bead therefore started releasing larger quantities of cells into the fermentation broth.
- DETD [0597] Continuous fermentations using the 50-L pilot scale gas-lift bioreactor loaded with LCC290 superflocculent yeast were performed over a 3-month period. The CO.sub.2 sparging gas was set at .about.2.5 mm/s. . .
- DETD . . . on a reactor working volume of 50 L. After the initial batch period, the cell concentration increased, reaching 3 billion cells/mL at about 750 hours into the fermentation (FIG. 6.65). This yeast mass then decreased to approximately 1 billion cells per mL at around 1000 hours and remained at this level until the end of the fermentation. Yeast viability was. . .
- DETD . . . runs were performed using the medium flocculent yeast strain LLC3021, as the immobilization matrix within the 50-L pilot scale gas-lift bioreactor. As with the two previous modes of immobilization, the initial yeast concentration was set at 4 g/L. This yeast was. . .
- DETD [0603] This initial batch stage allowed the yeast cells to flocculate and therefore be more easily retained within the gas-lift system. At the end of the batch startup, the. . . hours based on a reactor working volume of 50 liters. The yeast population (FIG. 6.70) increased to about 1 billion cells per milliliter and remained at this level for just over 1000 hours (between 500 and 1500 hours into the continuous. . . fermentation run). The yeast population doubled suddenly at .about.1500 hours into the fermentation and then leveled off at 2 billion cells/mL. This change in yeast population was unexpected. The yeast viability throughout the fermentation run was maintained at above 90% (FIG. . .
- DETD . . . were presented. All three carriers were deemed suitable for continuous primary fermentation within the 50-L pilot scale gas-lift draft tube bioreactor. Liquid residence times of 24 hours were

- achieved in all three cases. The fermentation runs using LCC290 superflocculent yeast reached. . .
- DETD [0608] The three fermentation systems seemed to reach a maximum free yeast cell concentration of .about.1 billion cells per milliliter. The inconsistency in yeast concentration impacted negatively on the .quadrature.-carrageenan system's ethanol productivity (lower initial pseudo-steady state ethanol. . .
- DETD . . . on the immobilization carriers that were viewed as potential alternatives for use in the 50-L pilot scale gas-lift draft tube bioreactor for fermentation. A total of 6 carriers--Chitopearl® chitosan beads, Celite® diatomaceous earth beads, Siran® glass beads, .quadrature.-carrageenan gel beads, medium. . .
- DETD . . . control the floc size during the fermentation process, and possible achieve increase mass transfer and therefore achieve further increases in bioreactor volumetric productivity.

TABLE 6.2

LCC3021

Comparison of several immobilization carrier for primary fermentation of beer within a gas-lift bioreactor system

Chitopearl ® Celite ® Siran ® Carrageenan LCC290

Goodbeer 3 1 1 4 5
4
Cost 2 3 1 3...

- DETD . . . more quickly (conversion of diacetyl to acetoin by yeast). It may also be possible that the non-agitated vessel had enough cells in suspension to further reduce diacetyl into the flavor inactive acetoin once the rate-limiting chemical conversion (1.sup.st step) had occurred.
- DETD [0624] The batch holding protocol was performed on liquid produced from the continuous fermentations in the 50-L gas-lift bioreactor using LCC290 superflocculent yeast, LCC3021 medium flocculent yeast or .quadrature.-carageenan immobilized yeast. The diacetyl reduction profiles of these three test. . .
- DETD . . . thesis. An acceptable beer with no major flavor defects can be produced using a 50-L pilot scale gas-lift draft tube bioreactor as the continuous primary fermentor when followed by a 2-day batch hold for the control of diacetyl. A minimum residence. . .
- DETD . . . technique utilized in this thesis allowed us to assess the mixing time and circulation rate within the 50-L pilot scale bioreactor during actual fermentations involving LCC290 superflocculent yeast, LCC3021 medium flocculent yeast and .quadrature.-carrageenan immobilized yeast. The mixing data was fit.
- DETD . . . of the production system designed, built and operated in the Labatt Experimental Brewery. The use of a gas-lift draft tube bioreactor with LCC290 superflocculent yeast and nitrogen as the sparging gas, followed by a 2-day batch hold at 21° C., is. . .
- DETD . . . continuous fermentations was flash pasteurized (Fisher Plate Heat Exchanger, combi-flow Type Eurocal 5FH) prior to feeding into the gas lift bioreactor and this wort was monitored regularly for microbial contaminants, as described in section 4.6. If contamination was detected in the. . .
- DETD [0659] Kappa-carrageenan gel X-0909 was a generous gift from Copenhagen Pectin A/S. Kappa-canrageenan gel heads containing entrapped lager yeast cells were produced using the static mixer process, as described in detail by Neufeld et al. (1996), with initial cell loadings of 10.sup.7-10.sup.8 cells/mL of gel, which are specified for

- each experiment. As illustrated in FIG. 4.1, the static mixer process is based on. . .
- DETD . . . yeast cell viability. The stain measures whether a yeast population is viable or non-viable based on the ability of viable cells to oxidize the dye to its colourless form. Non-viable cells lack the ability to oxidize the stain and therefore stain blue. Fink-Kuhles buffered methylene blue was prepared by mixing 500. .
- DETD . . . yeast solution was mixed with the methylene blue solution in a test tube, to a suspension of approximately 100 yeast cells in a microscopic field A small drop the well-mixed suspension was placed on a microscope slide and covered with a cover slip. Following one to five minutes of contact with the stain, the cells stained blue and the cells remaining colourless were enumerated. The percentage of viable cells was reported as a percentage of the total number of cells enumerated. Cell concentration was determined using a light microscope and a Hemacytometer (Hauser Scientific Company).
- DETD . . . containing 19 mL of distilled water. The beads were then disrupted using a Polytron® (Brinkmann Instruments) apparatus, to release the cells from the gel. Cell viability and concentration were then measured as described for the freely suspended cells.
- DETD . . . analyses. The wort that was used for continuous fermentations was also tested for contamination prior to transferring it into the bioreactor. To test for the presence of both aerobic and anaerobic bacteria, samples were plated on Universal Beer Agar (UBA, Difco Laboratories), with the addition of 10 mg/L of cycloheximide, and incubated. . .
- DETD . . . developed in our laboratory to ensure that the immobilized cell beads to be used for fermentations were free of contaminating bacteria before being pitched into the bioreactor. The main concern was to avoid contamination with beer spoilage organisms such as Pediococcus sp. and Lactobacillus sp. or wild. . . # 98139, NBB Broth Base, 0.02 g/L cycloheximide) is a semi-selective medium which is used to test for beer spoilage bacteria, such as Pediccoccus sp. and Lactobacillus sp. Copper sulphate broth (16 g/L YM broth, Difco; 0.4 g/L CuSO.sub.4) is a. . . Finally, Standard Methods (STA)+cycloheximide broth (16 g/L "Standard Methods" broth, Difco; 0 02 g/L cycloheximide) is used to test for bacteria found in water, wastewater, dairy products, and foods (Power and McCucn, 1988). The selective media were chosen to detect and. . .
- DETD [0672] Cultures were serially diluted to a suitable concentration of microorganisms, .about.100 cells/0.2 mL. for plating. The YPD plates were then incubated for approximately 3 days at 21° C. until yeast colonies were. . .
- DETD . . . Electron Microscopy (SEM) of Yeast Immobilized in Kappa-Carrageenan Gel Beads Kappa-carrageenan gel beads containing immobilized yeast were removed from the bioreactor through the sample port and placed in a 10 mL screw-cap glass vial, with the beads submerged in a small. . .
- DETD [0674] 4.9 Bioreactor Sampling Protocol
- DETD [0675] The bioreactor sample port (Scandi-Brew Type T Membrane Sample Valve) reservoir was filled with 70% (v/v) ethanol solution to maintain aseptic conditions. . .
- DETD . . . the top of the conical bases of the wort storage tanks, T-1 and T-2 (see section 4.2.1). A variable speed peristaltic pump provided volumetric flow rate of 11 L/hr through the dissolved oxygen analyzer block. ((Masterflex® L/S.TM. Digital Standard Drive, Cole-Parmer. . .
- DETD [0686] Dissolved Oxygen Measurement in the Bioreactor: Prior to performing the dissolved oxygen measurements on the

bioreactor, the Digox 5 analyser block was sanitized. The inlet of the sensor was connected to sterile, Tygon® Food Grade tubing. analyzer. The free ends of the tubing were then aseptically clamped to the 1/4" I.D. stainless steel ports on the bioreactor head plate and measurements were taken When the ports on the bioreactor were not in use, they were sealed using a short length of sterilized Tygon® food grade tubing. DETD [0687] Dissolved oxygen was measured on-line in the gas lift bioreactor by withdrawing liquid from the fermentation through a port situated on the bioreactor head plate. The fermentation liquid exited the bioreactor through a stainless steel filter (see section 4.1.2) connected to a 1/4 inch stainless steel pipe which penetrated the bioreactor head plate. The liquid then flowed through flexible Tygon® food grade tubing (1/4 inch id.) which was connected to a variable speed peristaltic pump (Masterflex® L/S Digital Standard Drive, Cole-Parmer cat. #P-07523-50), providing a volumetric flow rate of 11 L/hr through the dissolved oxygen analyzer block. The fermentation liquid was then recycled through a second quarter-inch stainless steel port, which penetrated the bioreactor head plate. Tygon® food grade tubing (Cole-Parmer, 1999) was used to connect the sensor to the bioreactor because of its supplier-specified low oxygen permeability of 30 cm.sup.3mm/(s.multidot.cm.sup.2.multidot.cmHg)+ 10.sup.-10 . The measurement was taken after 4-5 minutes of circulation. [0722] Chapter 5. Continuous Fermentation Using a Gas-Lift Bioreactor System [0723] A gas-lift draft tube bioreactor system was chosen for DETD continuous beer fermentation because of its published excellent mass transfer (liquid-solid) and mixing characteristics. Liquid-solid mass. . . encapsulated yeast. These bioreactors also provide good aeration, low power consumption, and are simple to construct. This has made gas-lift bioreactor systems very attractive for large scale operations, such as those used commercially for wastewater treatment (Driessen et al., 1997; Heijnen,. DETD [0724] 5.1 Gas-lift Draft Tube Bioreactor Description DETD [0725] This section gives a detailed description of the gas-lift bioreactor used in this work. DETD [0726] 5.1.1 Bioreactor Body DETD [0727] The 13 L (8 L working volume) gas-lift draft tube bioreactor designed for this work was a three phase fluidized bed (liquid/solid/gas) where the immobilized cells were kept in suspension by carbon dioxide gas driven internal liquid circulation (Heijnen, 1996) as shown in FIG. 5.1. A photograph of the bioreactor vessel is given in FIG. 5.2 and a detailed drawing with detailed dimensions is given in FIG. 5.3. Carbon dioxide and air flow into the bottom cone of the bioreactor through a sintered stainless steel sparger (CO.sub.2 purger nozzle, Part #9222, Hagedorn & Gannon, USA), 0.11 m length 0.013 m. . . outer diameter. Carbon dioxide was used as the fluidizing gas and air was used to supply oxygen to the yeast cells. [0728] A draft tube, concentrically located inside the columnar DETD bioreactor, functioned as the riser in this fluidized bed system while the outside annulus served as the downcorner. The internal draft. . was suspended from a cylindrical particle separator, seated on three stainless steel tabs in the expanded head region of the bioreactor. Keeping the draft tube and particle separator fittings inside the bioreactor, minimized the risk of microbial contamination from the outside environment. DETD [0729] Originally, the biorcactor had a mesh screen to separate the immobilized cells from the liquid at the outlet. However, the screen was prone to plugging, so a stainless steel cylinder was used.

annulus. The particles would hit the cylinder and fall back down into

the bulk liquid phase rather than leaving the bioreactor as overflow. Thus there was a small region near the bioreactor outlet that was free of immobilized cell particles. The bioreactor expanded head region also increased the surface area for gas bubble disengagement.

- DETD [0730] 5.1.2 Bioreactor Headplate
- DETD [0731] In FIG. 5.4 a schematic of the bioreactor headplate is given. Headplate ports were kept to a minimum to reduce the risk of contamination. The ports were either. . . controller system. The temperature controller gave feedback to a solenoid valve, which opened and closed the glycol supply to the bioreactor thermal jacket. Temperature was monitored using a thermometer (Cole-Parmer Waterproof Thermocouple thermometer, #90610-20) and a type T probe, which was welded into the bioreactor head plate. Dissolved oxygen was measured using a dissolved oxygen analyzer (Dr. Theidig, Digox 5), which required a flow of 9-11 L/hr of liquid broth through the analyzer block for accurate oxygen readings. Liquid was withdrawn from the bioreactor for oxygen measurement through a 1/4 i.d. pipe that went through the headplate into the fermentation liquid. As shown in. from the liquid, as it was pumped through the dissolved oxygen analyzer. The liquid was then returned back to the bioreactor through another 1/4" port in the headplate.
- DETD [0732] FIG. 5.5. Profile of liquid withdrawal port for oxygen sensor with filter unit submerged in bioreactor liquid phase.
- DETD [0734] The bioreactor was equipped with a membrane sample valve (Scandi-Brew®) welded into the bioreactor wall. The valve was designed for sampling under aseptic conditions. The membrane sealed directly against the fermentation liquid, allowing the. . . A small external reservoir of ethanol surrounded the membrane to maintain sterility between sampling. This valve was used for all bioreactor sampling and it was assumed that the composition of the liquid at the point of sampling was not significantly different than the composition of the liquid exiting the bioreactor outlet. As mentioned in the Materials and Methods chapter of this work, the bioreactor was sampled from a valve located on the outer wall of the bioreactor. In order to validate the assumption that the composition of the liquid exiting the bioreactor outlet was the same as the liquid sampled from the body of the bioreactor , mixing time studies were performed.
- DETD [0735] A pulse tracer method was used to determine mixing time in the gas-lift bioreactor (Chistie, 1989). A 1 mL volume of 10 N HCl was rapidly injected into the bioreactor annulus and the change in pH was logged over time, with timer, t=0 seconds at the time of the injection. . .
- DETD . . . 472 cm.sup.3/min (volumetric flow rate used throughout this work), and 661 cm.sup.3/min. In all three cases the pH in the bioreactor had equilibrated (.about.95% cutoff) in less than 2 minutes, as seen in Appendix 1. The mixing time was deemed to be sufficiently short to validate our original assumption that the bioreactor was well-mixed. This allowed us to assume that the composition of the liquid sampled from the bioreactor wall was not significantly different from that which flowed from the outlet, with an average liquid residence time of 24 hours in the bioreactor . From the appended figures, a definite liquid recirculation superimposed on mixing by dispersion was seen, which is typical of gas-lift. . .
- DETD . . . holding tanks (T-1 and T-2). During continuous fermentation the wort was transferred at a controlled flow rate to the gas-lift bioreactor (BR-1) containing immobilized yeast cells. Fermented liquid left the bioreactor as overflow and was collected into a receiving vessel (T-3). In the following sections, the operation of the continuous beer. . .

- DETD . . . Wort was held in these tanks at 2° C. for up to 2 weeks, supplying liquid to the continuously fermenting bioreactor, BR-1. At the end of the two-week period, the bioreactor feed was changed over so that wort was supplied from the second wort tank, which contained fresh wort. Two identical. . . wort. In all cases, wort was tested for contamination a minumum of two days prior to being introduced into the bioreactor (BR-1). If the wort was contaminated, it was discarded and fresh wort was immediately collected and pasteurized.
- DETD . . . the wort from chemical reactions with oxygen (Nazi β et al., 1993), to provide a consistent supply of wort to the bioreactor, and to minimize the risk of wort contamination with microbes during storage. The large 1,600 L (net) cylindroconical vessels (T-1. . .
- DETD . . . through a sterile gas filter situated at the top of the tank. Wort was then transferred using a variable speed peristaltic pump (P-1) (Masterflex® L/S.TM. Digital Standard Drive, Cole-Parmer cat #P-07523-50) to the 8 L bioreactor (BR-1) inlet using Norprene.TM. Food Grade L/S 16 flexible tubing.
- DETD [0747] 5.2.2 Continuous Fermentation Using Gas-Lift Draft Tube Bioreactor System
- DETD [0748] Wort was introduced near the bottom cone of the bioreactor, BR-1, through a 1/4" port. A mixture of filter-sterilized (Millipore, Millex®-FG.sub.50, 0.2 .quadrature.m Filter Unit), air and carbon dioxide (99.99% purity) flowed into the bioreactor through the sintered stainless steel sparger. A rotameter (R-3) was used to control the carbon dioxide flow rate at STP, . . . precalibrated mass flow controller (M-1) was used to control the flow rate of air at STP. Fermented liquid left the bioreactor as overflow and flowed through 1" I.D. reinforced PVC tubing into a 30 L stainless steel collection vessel (T-3) which. .
- DETD . . . foaming. This vessel also had a sterile gas filter, (Millipore, Millex®-FG.sub.50, 0.2 .quadrature.m Filter Unit), for gas release from the bioreactor (BR-1) and the collection vessel (T-3). The collection vessel was periodically emptied using a 1/4" valve V-12) situated 2" above. . .
- DETD . . and pressure of 45 psig, and circulated through cooling jackets for the wort holding tanks (T-1 and T-2), the gas-lift bioreactor (BR-1), and the product collection vessel (T-3). The two wort holding tanks and the bioreactor were equipped with liquid phase temperature probes which provided feedback to temperature controllers, which in turn controlled the flow of. . . glycol to the vessel jackets. The wort holding tanks stored the wort at 2° C., while the temperature within the bioreactor was controlled at temperatures of 12° C. to 22° C., depending upon the specific experiment. The product collection vessel did. . . was also used to jacket and cool the wort transfer lines from the wort tanks (T-1)and T-2) to the bioreactor (BR-1). Once the glycol had circulated through a given jacket, it was returned to a main line within the Pilot. . .
- DETD [0753] 5.3 Bioreactor Sterilization Protocol
- DETD [0754] The bioreactor (BR-1) was filled with a 2% (v/v) solution of Diversol ® CX/A (DiverseyLever, Canada), a sanitizing detergent, and soaked overnight. . . with cold water. This cycle of cleaning solution and water rinsing was repeated two times. In order to prepare the bioreactor for steam sterilization, the wort and gas lines were disconnected. The steam line was connected to the bioreactor inlet and the following valves were opened: the bioreactor inlet and purge valves (V-7, V-6), the gas inlet (V-17), product outlet valves (V-9, V-11), the membrane sampling valves (V-8, V-10), and collection vessel drain port (V-12). The plant steam valve was then slowly opened and the bioreactor valves were

adjusted so that a trickle of steam was observed at the exit of each external opening. After 60 minutes of steam exposure, all the external valves on the bioreactor were closed (V-17, V-8, V-10, V-12) except the wort bypass valve (V-6). When the steam valve was closed, the wort. . . filter was connected to the collection vessel to prevent contamination by non-sterile air entering the system as it cooled. The bioreactor gas line was also reconnected at V-17 as the plant steam line was closed in order to maintain a positive. . .

- DETD . . . into a 20 L stainless steel pressure vessel and heated in an autoclave for 45 minutes at 100° C. Immobilized cells were aseptically transferred into the cooled wort (40% v/v). The scaled vessel was transported to the Microbrewery Pilot Plant where the bioreactor system was housed. The 20 L vessel was connected to a quick connect fitting (Cornelius Anoka, Minnesota, USA), which was. . tubing (Cole-Parmer, USA). The other end of the PVC tubing was clamped to the membrane sampling valve (V-8) in the bioreactor wall. Filter-sterilized carbon dioxide was applied as 10 psig to the 20 L vessel and the membrane sample port was opened so that the immobilized cell mixture was transferred from the vessel into the bioreactor , without exposing die inoculum to the outside air environment. The internal components of the "quick connect" fittings of the 20 L vessel were removed to prevent plugging with immobilized cells upon transfer into the bioreactor. The cumulative particle size distribution (undersize) for the kappa-carrageenan gel beads is shown in FIG. 5.8. The arithmetic mean particle.
- DETD [0757] Following inoculation with immobilized cells, the bioreactor was operated in batch mode until the sugar and diacetyl concentrations reached targets of less than 3° Plato in terms. . . condensate in the line was replaced with fresh cold wort. At that point the bypass valve was closed and the bioreactor inlet valve (V-6) on the reactor was opened, commencing the continuous fermentation process.
- DETD . . . supplying wort from T-1, the continuous feed pump, P-1 was stopped and the valve (V-5) at the inlet of the bioreactor was closed. The wort transfer line was then connected to the second storage tank (T-2) and the line was flushed. . .
- DETD [0759] FIG. 5.6. Detailed equipment and flow diagram for continuous primary beer fermentation using a gas-lift bioreactor system (see Table 5.1 for detailed equipment description).

TABLE 5.1.

Detailed parts description for flow diagram shown in FIG. 5.6; PTFE, polytetrafluoroethylene; SS, stainless steel.

Item Description Size Mat'l Const.

BR-1 Bioreactor 8 L net 316 SS

T-1 Storage tank for wort 1600 L net 316 SS T-2 Storage tank for wort 1600 L net 316 SS T-3 Storage tank for beer 30 L net 316 SS

P-1 Pump (peristaltic, variable speed) for wort transfer <0.08 L/min SS rollers on Norprene ® Food flexible tubing from T-1 to BR-1

F-1 Filter for gas at outlet of T-1 $$^{<4}$\ bar polyprop.,. . .$

DETD [0762] Scientists have studied a variety of matrices for the physical entrapment of whole cells including calcium alginate (Bejar et al., 1992; Curin et al., 1987; Masschelein and Ramos-Jeunehomme, 1985; Nedovic et al., 1996; Shindo. . .

 ${\tt DETD}$. . cell colonization within kappa-ccarrageenan gel beads was

monitored over three cycles of repeated batch fermentation. The viability of the immobilized cells and the cells released into the liquid phase was examined. Fermentation parameters including ethanol, maltose, maltotriose, fructose, and glucose were followed throughout the repeated batch fermentations and then compared with control fermentations using only freely suspended yeast cells under the same nutrient conditions.

- DETD [0764] There has been little published information to date on the physical effects on cells after long term immobilization (Virkajarvi and Kronlof, 1998) and continuous exposure to external stresses and fermentation products. The second part of this chapter examines the viability, cell population distribution and physical appearance of yeast cells immobilized within carrageenan gel beads over an extended period of continuous fermentation in a gas-lift bioreactor. Also examined over extended periods of time were the relative percentage of respiratory deficient yeast in the immobilized and freely suspended cell population of the bioreactor.
- DETD . . . ions, and will undergo some syneresis. The increased gel strength afforded by kappa-carrageenan makes it desirable for immobilizing whole yeast cells.
- DETD . . . controlled such that it is high enough to be a gel under fermentation conditions, yet low enough that the yeast cells may be mixed with the carrageenan in its sol state without detrmiental effects on viability prior to bead gelification.
- DETD . . the need for further study on the effects of immobilization within gel matrices on yeast cell metabolism and physiology. Immobilized cells are not subjected to the same micro-environment as the free cells in the liquid phase because there are additional barriers from the gel matrix and other entrapped yeast cells which must be surmounted, before substrates can be transported to their surfaces (FIG. 6.3). There have been many studies on. . . 1995; Venancio and Ticxicra, 1997) to gain a better understanding of the potential negative effects that nutrient limitation to immobilized cells may have on fermentation performance. The effective diffusivities of small molecules within carrageenan gel are comparable with the diffusivities of. . Stephanopoulos, 1986). Once the nutrients enter the beads, transport is relatively slow because molecular diffusion dominates. This means the yeast cells at the periphery of the gel beads may have a distinct nutritional advantage over those in the center of the beads. The age of the immobilized yeast must also be considered. Entrapped cells age as a continuous fermentatuon proceeds over the course of months and they ferment under a defined set of pseudo-steady-state conditions. However, during batch fermentation, yeast cells are exposed to an environment that changes with time and the cells are only reused for a limited number of fermentations before disposal. More research is needed to study the long-standing effects. . .
- DETD . . . within the gel bead and yeast cell morphology were examined. Scanning electron microscopy (SEM) was used to examine kappa-carrageenan-immobilized yeast cells in different regions of the gel bead at four different times: 1) immediately after bead production; 2) after two days of batch fermentation; 3) after two months of continuous fermentation in a pilot scale gas lift bioreactor; 4) after six months of continuous fermentation in a pilot scale gas lift bioreactor. Yeast viability and concentration in both immobilized and liquid phase cells were also measured. Also examined was the relative percentage of respiratory deficient yeast (immobilized and free cells in the liquid phase) after five months of continuous fermentation in the gas lift bioreactor and this was compared with the percentages found in traditional batch beer fermentations. A production lager yeast strain was used. . .

DETD . . . Bead Production: kappa-carrageenan gel X-0909 was a generous

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gift from Copenhagen Pectin A/S. Kappa-carrageenan gel beads contained entrapped lager yeast cells were produced using the static mixer process with an initial cell loading of 2.6+10.sup.7 cells/mL of gel (U.S. patent application Ser. No. 2,133,799 (Neufeld et al. 1996) and a bead diameter of 0.5 to 2.0. .
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- DETD . . . fermentations were conducted with freely suspended cell control fermentations, which were conducted under the same conditions except that only free cells were pitched into the fermentations at a rate of 4 g/L. Samples were analyzed for free and immobilized cell viability. . .
- DETD [0777] Ethanol productivity, V.sub.ethanol, the amount of ethanol produced per total bioreactor working volume per unit fermentation time was calculated using equation 3.25 for R1, R2 and R3, and the free cell. . . complete. In the case of the yield factors and ethanol productivity, the contributions of the immobilized and freely suspended yeast cells were not distinguished from one another.
- DETD . . . gas lift draft tube bioreactors were used for continuous fermentations. All data collected were from an 8 L working volume bioreactor, except the 2 month scanning electron micrographs, which were collected from a 50 L bioreactor using the same fermentation medium and immobilization method. Immobilized cell beads at 40% (v/v) were fluidized within the bioreactors using . .
- DETD [0782] Microbiological Analyses: Samples were taken from the liquid phase of the gas lift bioreactor at least once a week to test for contaminants including wild yeast, non-lager yeast, and aerobic and anaerobic beer spoilage bacteria. After five months, the liquid phase yeast cells were assayed in duplicate for respiratory deficient mutation.
- DETD [0783] Scanning Electron Microscopy (SEM): Kappa-carrageenan gel beads (1.0-1.5 mm diameter) containing immobilized lager yeast cells were sampled for SEM examination at four different times: 1) after immobilized cell bead production and before inoculation of beads. . . 2 days in batch fermentation; 3) after 2 months of continuous fermentation in a pilot scale gas lift draft tube bioreactor; 4) after 6 months of continuous fermentation in a pilot scale gas lift draft tube bioreactor. The methodology for used for SEMs and the related sample preparation are described in section 4.7.
- DETD [0787] Fermentation time was greatly reduced each time the immobilized cells were repitched into fresh wort, as seen in FIGS. 6.4(a), (b), and (c) illustrating maltose, maltotnose, glucose, fructose and ethanol. . .
- DETD . . . 6.5. Maltose, maltotriose, glucose, fructose, and ethanol concentration versus fermentation time for freely suspended lager yeast control fermentations (no immobilized cells).
- DETD . . . concentrations respectively versus fermentation time of R1, R2 and R3. During repeated R1, maltose was taken up by the yeast cells almost immediately after pitching into fresh wort. Ethanol concentrations reached their peak earlier in repeated R1 and also reached higher. . . fermentations. As shown in FIG. 6.6(b), the initial lag in ethanol production in R1 was drastically reduced when these immobilized cells were repitched in R2 and further reduced after repitching for R3.
- DETD [0790] FIG. 6.6(b) Ethanol concentration verses fermentation time for repeated batch fermentations, R1, R2, and R3 using lager yeast cells immobilized in kappa-carrageenan gel beads.
- DETD [0791] FIG. 6.7(a) shows immobilized cell concentration per total bioreactor volume vs. fermentation time for R1, R2 and R3. The free cells released from the immobilized cell matrix into the bulk liquid phase in these fermentations vs. time are shown in FIG. 6.7(b). In FIG. 6.7(c) the total of immobilized and free yeast cells per total reactor volume are shown for the three batches. FIG. 6.7(a) shows that the concentration of immobilized cells

within the kappa-carrageenan gel continued to increase following their initial innoculation into wort for R1. When the beads were repitched. into fresh wort for repeated R2, growth continued to occur within the gel beads. The third time that the encapsulate cells were repitched into fresh wort, the rate of increase in immobilized cell concentration had slowed. The concentration profile of free cells released from the kappa-carrageenan gel matrix into the bulk liquid phase, immobilized cells, and total cells in the fermentation for R1 is shown in FIG. 6.8.

DETD . and liquid phase) cell concentration verses fermentation time for R1, the first of three repeated batch fermentations using lager yeast cells immobilized in kappa-carrageenan gel beads.

DETD . within the kappa-carrageenan gel bead was increasing at a similar rate to the control fermentation, which contained only liquid phase cells. This was confirmed by comparing the average growth curve of the free cell fermentations in FIG. 6.9 to the similar growth curve cells of immobilized in carrageenan in R1 in FIG. 6.10. During R1, the gell beads were not yet fully colonized and. effect on yeast cell growth within the beads. By R2, the matrix appeared to be restricting the growth of the cells within the gel bead, as indicated by a smaller increase of cell number during this fermentation cycle. This could be due to the nature of the gel or the crowding of the yeast cells within the beads, or to a lack of nutrient supply to the cells.

DETD . . . ethanol from substrate fermentable sugars, Y.sub.P/S, are shown for the three batch generations and the control. In Table 6.2, the bioreactor volumetric productivities of ethanol are also given, calculated using the data given in Table 6.1. The yields of ethanol from. . . yield of 0.51 predicted from the Guy-Lussac equation. As mentioned earlier, biomass production and other by-products formed by the yeast cells prevent efficiencies from reaching higher than 95% of theoretical (Hardwick, 1995). The volumetric bioreactor productivity of ethanol in the three repeated batch fermentations varied significantly from batch to repeated batch. Ethanol productivity increased with each cycle of repeated batch fermentation and, by R3, the immobilized cells were more productive than the control fermentation. The total amount of ethanol produced in R2 was not significantly greater than. . . R1 and of the control fermentation. There are many factors that could contribute to this increased fermentation rate of immobilized cells with each batch repetition, such as yeast cell adaptation to the fermentation conditions and the progressively increasing cell concentration. The total number of cells per bioreactor volume only becomes significantly greater than that of the control by R3. In FIG. 6.7(b), the graph of freely suspended cell (released from the gel matrix) concentration in the bulk liquid vs. fermentation time demonstrated that the number of cells released from the gel beads increased with each batch generation. Once the beads became more fully loaded with yeast cells, they appeared to release more cells into the bulk liquid phase. Husken et al. (1996) conducted studies that examined bacterial cell colony expansion and eruption/release from kappa-carrageenan gel slabs. Vives et al. (1993) have reported that the maximum concentration of yeast cells they have achieved in kappa-carrageenan gel beads was 10.sup.9 cells per gram of gel, which is the concentration that was reached within the gel particles by R2. Similar maximum cell. .

DETD [0795]

TABLE 6.2

(m.sup.3 bioreactor volume .multidot. h)] for immobilized cell batch fermentations (R1, R2, and R3) compared with freely suspended cell batch fermentations.

Fermentation V.sub.Ethanol(kg/m.sup.3 hr)*

DETD [0796] Another factor affecting the increased bioreactor volumetric productivity observed with each repeated batch fermentation, involves yeast cell adaptation. By the end of the first fermentation, yeast cells had adapted their metabolic machinery to the given fermentation conditions. This may result in a decrease in the lag phase.

. . with freshly prepared lager yeast. It would be interesting to repitch the freely suspended control yeast alongside the repitched immobilized cells to further examine this effect relative to the cell concentration effects.

DETD . . . 6.11 indicates that immobilized cell viability, using the methylene blue method as an indicator, was low (<50%) when the immobilized cells were initially pitched into wort in R1, but the viability of immobilized cells was above 90% after 48 hours of fermentation. The yeast cells rapidly colonized the beads, and viability remained high throughout R3. However by repeated R3, viability tapered off slightly toward the end of the fermentation. However, throughout all three repeated batch fermentations, the free cells that were released into the bulk liquid medium had higher viability than their immobilized counterparts. The immobilization matrix may have a negative effect on yeast cell viability (mass transfer limitations and/or spatial limitations), or viable yeast cells may be preferentially released from the immobilization matrix into the bulk liquid medium over non-viable cells.

DETD . . . fermentation time, is given in FIG. 6.12. The slope is equal to the local maximum specific growth rate of the cells at 21° C. in brewer's wort, with shaking at 150 rpm. The local maximum specific growth rate of the yeast. . .

DETD . . . exposed to fermentation medium, and following immobilized cell bead production using the static mixer process, the cell concentration was 2.6+10.sup.7 cells/mL of gel bead (Table 6.3, where values are the averages of two samples). SEM imaging shows the cells to be individually and uniformly distributed throughout the gel bead (FIG. 6.13).

TABLE 6.3

Viability (methylene blue) and concentration of freely suspended and immobilized lager yeast cells entrapped in kappa-carrageenan gel beads over fermentation time.

Freely Suspended Immobilized Yeast in Yeast. Gel Phase In Liquid Phase Cell Conc. Via- Cell Conc. Fermentation Viability (cells/mL bility cells/mL Time Mode (%) in liquid) (%) n/a 2.6E+07 n/a n/a n/a 5.5E+07 92 2.35E+08 2 days Batch 98 Continuous. . . 2 months DETD

. . . was >90% following 2 days of batch fermentation, and cell concentration within the gel bead had increased ten-fold (Table 6.3). Cells (>90% viable) had also begun to be released from the gel into the bulk liquid phase of the fermentation, yielding a concentration of 10.sup.7 cells/mL of liquid. Small yeast colonies formed inside the gel beads, with many bud scars present on individual

cells as seen in FIG. 6.14.

- DETD [0802] Immobilized yeast cell viability decreased after 2 months of continuous fermentation in a gas lift bioreactor (Table 6.3), but the cells in the bulk liquid phase remained highly viable (>90%), and this finding was supported during several different continuous fermentations in. . . to the yeast positioned at the center of a gel bead was made in several samples using SEM imaging. The cells located toward the periphery of the beads were ovoid and smooth with many bud scars (FIG. 6.16), indicative of yeast multiplication (Smart, 1995). The cells that were imaged at the center of the bead (FIG. 6.17) appeared malformed and displayed little evidence of bud scar. . .
- DETD . . immobilized within the carrageenan gel had dropped to below 50% after six months of continuous fermentation in the gas lift bioreactor, (Table 6.3). It should be noted that while only a single data point for immobilized cell concentration and viability was collected at six months, data at the five-month mark was similar, with an immobilized cell concentration of 1.14+10.sup.9 cells /mL of gel and viability of <50%. While a gradual decline in immobilized cell viability was seen over times the viability of the cells in the bulk liquid phase remained reliably high. In addition, even though immobilized cell viabilities were low in the beads as a whole, the bioreactor produced a fully fermented beer during its sixth month of continuous operation. Possible reasons for this finding include the significant contribution of the highly viable freely suspended yeast cells to the fermentation. As well, there is the potential contribution of viable immobilized cells located at the periphery of the gel bead where there are fewer barriers to mass transfer, as compared to the cells located at the center of the bead. It is unclear whether the immobilized cells had the ability to redistribute themselves within the gel matrix, or if these cells remained stationary where they were first located. A concentration of 10.sup.9 cells/mL of gel bead was the maximum reached within these beads over the six-month period of continuous fermentation.
- DETD . . . cavity was not observed in fresh bead preparations. Previous work by others (Bancel et al., 1996) has shown that growing cells induced weakening of the gel network Audet et al. (1988) reported that the addition of locust bean gum to kappa-carrageenan modified the mechanical strength of gel beads for the immobilization of bacteria.
- DETD [0805] Over the entire six month beer fermentation experiment, the gas lift bioreactor was tested a minimum of once a week for contamination. No bacterial contaminants were detected at any time during the. . . of growth on PYN medium at 37° C., but did not grow aerobically or anaerobically on DUBA medium (selective for bacteria), did not ferment dextrins, and showed no growth on CuSO.sub.4 medium (selective for wild yeast).
- DETD [0806] After five months, the average percentage of respiratory deficient yeast cells was 7%, which is higher than what is normally found using this strain during industrial batch fermentations (2% average). Other. . .
- DETD . . . nuclear magnetic resonance (NMR) spectroscopy (Fernandez, 1996) and confocal microscopy (Bancel and Hu, 1996) have been used to examine immobilized cells non-invasively. NMR imaging techniques have allowed researchers to study transport, flow and spatial distribution of cells and biochemicals in biofilms. Researchers (Bancel and Hu, 1996) have also shown that confocal laser scanning microscopy can be used to observe cells immobilized in porous gelatin microcarriers through serial optical sectioning.
- DETD . . . (Mochaba et al., 1998). It measures whether a yeast population is viable or non-viable based on the ability of viable cells

to oxidize the dye to its colourless form. Non-viable cells lack the ability to oxidize the stain and therefore remain blue (O'Connor-Cox et al., 1997). Plate count and slide culture techniques are based on the ability of the cells to grow and produce macrocolonies on agar plates or microcolonies on media covered microscope slides (Technical Committee and Editorial Committee. . . methods as well as developing the confocal microscopy technique using vital staining. In addition to measuring the viability of the cells, the issue of "vitality" of the immobilized cells must also be addressed in future work. Where viability has been used to describe the ability of cells to grow and reproduce, vitality measures yeast fermentation performance, activity, or the ability of the yeast to recover from stress. . .

- DETD [0812] Using continuous fermentation to produce beer is very different from other applications using immobilized cells because the resulting product is not measured in terms of one component of interest such as ethanol. Rather, it is. . .
- DETD [0813] 7.1.1 Effect of Relative Amounts of Air in the Bioreactor Fluidizing Gas on Yeast Metabolites During Primary Continuous Fermentation
- DETD [0814] The amount of air and hence oxygen in the bioreactor fluidizing gas was varied while residence time, temperature and all other controllable process variables were held constant. The total volumetric. . . kappa-carrageenan gel beads containing immobilized LCC 3021 yeast were used throughout the trial with an initial cell loading of 1+10.sup.8 cells/mL of gel. Four different volumetric flow rates of air were imposed on the system throughout the trial (Table 7.1), and the average bioreactor residence time, R.sub.t, was 1.18 days.

TABLE 7.1

Air volumetric flow rates supplied to the bioreactor through the sparger during continuous fermentation. The total volumetric flow rate supplied to the bioreactor was 472 mL/min at STP, with carbon dioxide making up the remainder of the gas. Air Volumetric Percent Air Flow Rate in. . .

- DETD . . . glucose), ethanol, total total diacetyl, beer volatiles (selected esters and alcohols), and liquid phase yeast cell concentration and viability. The bioreactor was also tested for contamination a minimum of once a week.
- DETD [0816] The dissolved oxygen concentration in the bulk liquid phase of the bioreactor was measured when the continuous fermentation was assumed to be at pseudo-steady state for each volumetric flow rate of air. . .
- DETD [0818] Even when the amount of oxygen in the bioreactor fluidizing gas was relatively low (34 mL/min at STP), the concentrations of acetaldehyde and total diacetyl found during the experiment. . . for the North American lager beer market. Therefore a novel approach was taken, where liquid taken from the continuous primary bioreactor was held in batch for 48 hours at a slightly elevated temperature of 21° C. to reduce the concentration of . . .
- DETD [0819] Continuous primary fermentation was performed in a 50 L gas lift bioreactor using a highly flocculent variant of the LCC3021 yeast strain for this trial because the sample volume requirement for the study was too large relative to the volume of the 8 L bioreactor. Operating conditions were 1180 mL/min CO.sub.2 and 189 mL/min air at STP in the fluidizing gas, an average bioreactor residence time, R.sub.t, of 1.0 day, a temperature of 15° C., and high-gravity 17.5° P lager brewer's wort.

- DETD . . . a 3 inch, 16 gauge needle, sanitized using a 70% (v/v) ethanol solution, was used to withdraw sample from the bioreactor by puncturing the septum of the membrane of the sample valve and the sample was injected into the 100 mL. . . pressure within the vial during filling. The aerobic samples were exposed to the atmosphere as they were drained from the bioreactor by fully opening the membrane sample valve into the 100 mL unsealed sample vials, without using a syringe and needle.
- DETD . . . to allow the yeast to settle out of solution, leaving a cell concentration in the bulk liquid of approximately 10.sup.6 cells /mL. Once settled, the liquid from each 100 mL vial was decanted into three 25 mL vials. The anaerobic samples were. . . sample. Samples were collected for analysis at 2, 24, and 48 hours. A sample was also taken directly from the bioreactor and analyzed immediately in order to assess the state of the fermentation within the bioreactor at the time of the protocol. The samples were analyzed for total fermentable carbohydrate (as glucose), ethanol, total diacetyl, and. . .
- DETD . . on yeast metabolic activity, an experiment was performed in which a step change in wort volumetric flow rate to the bioreactor was imposed during continuous primary beer fermentation using LCC3021 yeast cells immobilized in kappa-carrageenan gel beads. The bioreactor temperature was held constant at 17° C. throughout the trial. The gas volumetric flow rate supplied to the bioreactor was also constant at 472 mL/min at STP, The gas was a mixture of air (11 mL/min at STP) and carbon dioxide (461 mL/min at STP). The initial concentration of yeast cells in the kappa-carrageenan gel was 2.6+10.sup.7 cells/mL of gel bead and the bioreactor contained 40% (v/v) of beads. The following analyses were performed repeatedly throughout the trial: carbohydrates, free amino nitrogen (FAN), total. . . (as glucose), ethanol, total diacetyl, beer volatiles (selected esters and alcohols), and liquid phase yeast cell concentration and viability. The bioreactor was also tested for contamination, a minimum of once a week.
- DETD . . . sugars are not being introduced. In the continuous fermentation system a constant low level of oxygen is supplied to the bioreactor through a sparger, and fresh wort is continuously supplied to the bioreactor. Therefore, a novel strategy using a commercial enzyme preparation was explored to control diacetyl concentration in the continuous bioreactor
- DETD [0828] Alpha-acetolactate decarboxylase was added to the wort fed into the bioreactor in order to examine its net effect on total diacetyl concentration. Other strategies for reducing diacetyl, including a batch warm. . . success in reducing diacetyl levels post fermentation, but neither addresses the level of diacetyl at the source (i.e. at the bioreactor outlet). By using ALDC in the wort to reduce the diacetyl concentration coming out of the bioreactor , the post-fermentation treatment periods could be minimized or eliminated.
- DETD . . . 4.1.1.5) from Bacillus brevis, produces the enzyme ALDC.
 Because ALDC is an enzyme that is produced by a genetically modified organism (GMO), there are public perception issues that would need to be addressed before using such an enzyme in a commercial. .
- DETD . . . the Labatt London brewery. Ethanol, total fermentable carbohydrate (as glucose), total diacetyl, and liquid phase cell concentration were monitored. Yeast cells were immobilized in kappa-carrageenan gel beads as described in the Chapter 4. The bioreactor was allowed three turnover times, before it was assumed to have reached pseudo-steady state. As mentioned earlier, the diacetyl method. . .
- DETD [0833] Continuous Fermentation Conditions: Continuous fermentations were

performed in the 8 L gas lift draft tube bioreactor pitched at 40% (v/v) with kappa-carrageenan gel beads containing immobilized lager yeast cells. The bioreactor was sparged with a mixture of carbon dioxide (438 mL/min at STP) and air (34 mL/min at STP). Fermentation temperature was controlled at $15^{\,\rm o}$ C. throughout the trials and the bioreactor residence time, R.sub.t, was 1.5 days. Total diacetyl concentration was monitored under these conditions and an average pseudo-steady state control. . . was then added to the wort at a concentration of 72 .quadrature.g/L (108 ADU/L) and total diacetyl concentration in the bioreactor was monitored for a response.

- DETD . . . minutes at 100° C. The wort was held at 2° C. in a controlled temperature water bath while feeding the bioreactor . Once a pseudo-steady state total diacetyl concentration had been reached within the bioreactor, 72 .quadrature.g/L (108 ADU/L) of ALDC was added to the wort inside the 20 L vessel. The initial biomass loading in the kappa-carrageenan gel beads was 3+10.sup.7 cells/mL of gel.
- DETD . . . the brewhouse into a 20 L stainless steel vessel, and autoclaved for 45 minutes at 100° C. While feeding the bioreactor, the wort was held at 2° C. in a controlled temperature water bath. The initial biomass loading in the kappa-carrageenan gel beads was 3+10.sup.7 cells/mL of gel. Once a pseudo-steady state total diacetyl concentration had been reached within the bioreactor, 72 L .quadrature.g/L (108 ADU/L) of ALDC was added to the wort inside the 20 L vessel.
- DETD . . . maintain a constant dissolved oxygen concentration of <0.10 mg/L, as described in Chapter 5. The wort was fed into the bioreactor from this tank until a pseudo-steady state total diacetyl concentration was reached. ALDC (72 .quadrature.g/L) was then aseptically added to. . . held enough ALDC dosed wort to complete the trial. The initial biomass loading in the kappa-carrageenan gel beads was 10.sup.8 cells/mL of gel.
- DETD [0838] 7.2.1 Effect of Relative Amounts of Air in the Bioreactor Fluidizing Gas on Yeast Metabolites During Primary Continuous Fermentation
- DETD . . . acetate, 1-propanol, isobutanol, isoamyl acetate, isoamyl alcohol, ethyl hexanoate, and ethyl octanoate concentrations are plotted versus continuous fermentation time. All bioreactor operating conditions were held constant throughout the protocol except the percentage of air in the bioreactor sparging gas, which is marked directly on the figures. In Table 7.2 the averages for each analyte at pseudo-steady state. . . a minimum of three reactor turnover times) are summarized.

TABLE 7.2

Summary table of effect of air volumetric flow rate to the bioreactor through the sparger on liquid phase yeast and key yeast metabolite concentrations in the bioreactor at a residence time, R.sub.l, of 1.18 days, averages at pseudo-steady state.

Average* Analyte Air Volumetric Flow Rate (mL/min)

Concentration		94	354	34
Cell Conc (cells/mL) Total Ferm. Glucose		3.87E+08 1.36	2.98E+08 1.25	4.73E+08 2.07
(g/100 mL)				_ , ,
FAN (mg/L)		196.9	171.7	162.8
Ethanol $(g/100 \text{ mL})$		6.14	5.46	5.74
Total diacetyl (ug/L).	•	•		

 ${\tt DETD}$. . . experiment. The flavour compounds that were studied in this

work were produced by a combination of free and immobilized yeast cells and the relative contributions from each source were not determined. There was more than one source of freely suspended yeast cells in this work: biomass growth and cells that were released from the gel beads into the bulk liquid medium. Research has shown with compound models of cell release and growth, that when cells are being released from biofilms, even if the bioreactor is operated high dilution rates, there will still be a population of cells in the output liquid (Karamanev, 1991)

- DETD . . . Liquid phase yeast viability versus relative continuous fermentation time. The volumetric flow rate of air at STP supplied to the bioreactor through the sparger is indicated an the graph. The remainder of the gas was carbon dioxide and the total volumetric.
- DETD . . . did not coincide with maximum ethanol concentration or minimum total fermentable carbohydrate (as glucose) concentrations. The ethanol concentration within the bioreactor liquid phase decreased while total fermentable carbohydrate (as glucose) increased when the volumetric flow rate of air in the sparge. . .
- DETD . . . carbohydrate (as glucose) concentration versus relative continuous fermentation time. The volumetric flow rate of air at STP supplied to the bioreactor through the sparger is indicated on the graph. The remainder of the gas was carbon dioxide and the total volumetric. . .
- DETD . . . considered an undesirable flavour compound in beer, one of the main reasons to optimize the amount of oxygen in the bioreactor is to control levels of this flavour compound. After the 354 mL/min air phase, the flow rate was dropped to. . .
- DETD . . . Liquid phase acetaldehyde concentration versus relative continuous fermentation time. The volumetric flow rate of air at STP supplied to the bioreactor through sparger is indicated on the graph. The remainder of the gas was carbon dioxide and the total volumetric gas. . .
- DETD . . . The concentration of each ester rose and then tapered off, as the liquid phase cell concentration decreased rapidly in the bioreactor.
- DETD . . . and ethyl octanoate concentration versus relative continuous fermentation time. The volumetric flow rate of air at STP supplied to the bioreactor through the sparger is indicated on the graph. The remainder of the gas was carbon dioxide and the total volumetric.
- DETD . . . 7.11. Liquid phase 1-propanol concentration versus relative continuous fermentation time. The volumetric flow rate air at STP supplied to the bioreactor through the sparge is indicated on the graph. The remainder of the gas was carbon dioxide and the total volumetric. . .
- DETD . . . to limit their production is important. As discussed in the literature review, when the supply of oxygen to the yeast cells is increased, there is enhanced anabolic formation of amino acid precursors and thus an overflow of higher alcohols, oxo-acids, and.
- DETD [0853] For the bioreactor conditions used in this experiment, the pseudo-steady-state (after a minimum of three reactor turnover times) dissolved oxygen concentrations measured in the liquid phase of the bioreactor were close to zero (less than 0.03 mg/L).
- DETD . . . air flow rate (354 mL/min). This is because the physiological state of the yeast resulting from the exposure to previous bioreactor conditions, the immobilization matrix and continuous fermentation time may also have caused other changes in flavour production.
- <code>DETD</code> [0855] No contamination was detected in the bioreactor at any

- point during this experiment. In order to balance the requirement of yeast for some oxygen to maintain yeast. . .
- DETD [0861] In FIG. 7.14 the aerobic samples showed an early increase in acetaldehyde upon exposure to aerobic conditions outside the bioreactor. The combination of aerobic conditions, with sugar consumption and ethanol production, could account for this result. By the end of. . .
- DETD . . . concentration versus post fermentation hold time for aerobic and anaerobic treated samples after continuous primary fermentation in a gas lift bioreactor. Error bars represent the upper and lower limits of the experimental data (n=2).
- DETD . . . concentration versus post fermentation hold time for aerobic and anaerobic treated samples after continuous primary fermentation in a gas lift bioreactor. Error bars represent the upper and lower limits of the experimental data (n=2)
- DETD . . . concentration versus post fermentation hold time for aerobic and anaerobic treated samples after continuous primary fermentation in a gas lift bioreactor. Error bars represent the upper and lower limits of the experimental data (n=2).
- DETD . . . the ideal scenario will be to eliminate the secondary holding period entirely by optimizing the conditions in the primary continuous bioreactor. However, further gains can be made using the holding period, by optimizing the holding temperature (diacetyl removal by yeast is. . .
- DETD [0870] Volumetric beer productivity calculations are given in Appendix 3. The process described in this section, with a continuous bioreactor operating with a 24 hour residence time followed by a 48 hour batch hold, is 1.8 times more productive than. . . more susceptible to microbial contamination (i.e. high sugar concentration, temperature, and oxygen, with low concentrations of ethanol). In the gas-lift bioreactor system presented in this work, the bioreactor has a low fermentable sugar concentration, low pH, high ethanol concentration, and low concentrations of oxygen, making the environment inhospitable. . .
- DETD [0872] FIGS. 7.23-7.28 show the analytical results obtained from the bioreactor liquid phase. In Table 7.3, the average concentrations and flow rates of the measured analytes at pseudo-steady state (after a minimum of three bioreactor turnover times) are listed at the two liquid residence times used during this experiment. While liquid phase yeast viability did not change significantly when the flow rate of wort to the bioreactor was increased, the concentration of yeast cells did change as seen in FIG. 7.23. Table 7.3. (a) Summary table of effect of bioreactor residence time on liquid phase yeast and key yeast metabolite concentrations, averages at pseudo-steady state; (b) Summary table of the effect of bioreactor residence time on liquid phase yeast and key yeast metabolite flow rates at the bioreactor outlet averages at pseudo-steady state.

Bioreactor Residence Time

	1.8 days	0.9 days
(a) Average Analyte Concentration		
Cell Conc (cells/mL)	2.38E+08	1.32E+08
Tot. Ferm. Glucose (g/100 mL)	0.29	6.09
FAN (mg/L)	106.3	246.4
Ethanol (g/100 mL)	5.16	4.80
Total Diacetyl 22.78	9.13	
Isoamyl Acetate (mg/L)	0.90	1.28
Isoamyl Alcohol (mg/L)	76.67	51.39

(b) Average Analyte

Flow Rate

- DETD [0873] FIG. 7.23. Liquid phase yeast cell concentration versus relative continuous fermentation time, effect of liquid residence time in bioreactor. R.sub.t is bioreactor liquid residence time in days.
- DETD . . . 7.4, the consumption rate of total fermentable carbohydrate (as glucose) increased while free amino nitrogen consumption rate decreased, with decreasing bioreactor residence time. The yield factor, Y.sub.P/S, of the fermentation product ethanol from fermentable glucose substrate, increased from 0.3 to 0.5. . . spectroscopy technique (GC-MS), that beer flavour volatiles including ethanol, acetaldehyde, ethyl acetate, and isoamyl acetate are detected in the gas-lift bioreactor headspace during continuous fermentation.
- DETD . . . 7.25. Liquid phase free amino nitrogen and 1-propanol concentration versus relative continuous fermentation time, effect of liquid residence time in bioreactor. R.sub.t is bioreactor liquid residence time in days.

TABLE 7.4

Mass balances on free amino nitrogen and total fermentable carbohydrate (as glucose) based on average data. . .

- DETD . . . FIG. 7.26. Liquid phase total diacetyl and acetaldehyde concentration versus relative continuous fermentation time, effect of liquid residence time in bioreactor. R.sub.t is bioreactor liquid residence time in days.
- DETD [0879] FIGS. 7.25 and 7.27 show the effect of decreasing bioreactor residence time on the liquid phase concentrations of the higher alcohols 1-propanol, isobutanol and isoamyl alcohol. All three higher alcohols decreased in concentration when the bioreactor residence time was decreased.
- DETD . . . FIG. 7.27. Liquid phase isobutanol and isoamyl alcohol concentration versus relative continuous fermentation time, effect of liquid residence time in bioreactor. R.sub.t is bioreactor liquid residence time in days.
- DETD . . . for an increase in liquid phase cell growth without increasing the oxygen supply to the system, the conditions in the bioreactor promoted ester production. Hough et al. (1982) state that increased growth and decreased oxygen conditions encourage ester formation.
- DETD [0883] Experiment 1: The bioreactor was contaminated with aerobically growing Gram positive cocci before the trial could be completed. It was determined that the bioreactor itself was contaminated, since microbiological testing of the wort supply showed no contamination. This pointed to the need for bioreactor upgrades with improved safeguards against contamination. However, before the system was shut down, a decrease in total diacetyl concentration was. . . wort supply. Unfortunately it was not possible to draw any conclusions from this data due to the confusing effects of bioreactor contamination.
- DETD [0884] Experiment 2: As a result of numerous bioreactor upgrades, the system operated without contamination throughout the duration of Experiment 2. The data for this experiment is given in. . . of ALDC to the wort, which makes the use of this enzyme promising for the future (averages taken after three bioreactor turnover

times.) As seen in FIGS. 7.30 and 7.31, total fermentable carbohydrate (as glucose) and cell concentration drifted slightly during this trial, which may have been caused by slight differences in the wort, supplied to the bioreactor before and after the addition of ALDC.

DETD . . . pseudo-steady state total diacetyl concentration before and after the addition of ALDC to the wort supply (averages taken after three bioreactor turnover times). No contamination was detected at any point during this experiment. The concentration of total diacetyl was reduced by. . .

DETD . . . economics of using ALDC for diacetyl reduction during gas lift continuous fermentations will depend on the optimum enzyme dosage under bioreactor conditions and the amount of time saved by its use.

TABLE 7.5

Summary of average pseudo-steady state effect of ALDC addition to wort fermentation medium on total diacetyl concentration during continuous beer fermentation in a gas lift bioreactor.

Average Total Diacetyl Percent Concentration (.quadrature.g/L) (ALDC, Diacetyl Experiment (ALDC absent) 60.quadrature.L/L) Reduction Experiment II 495 260 47 Experiment III 445 245 45

*averages. . .

DETD [0890] The foregoing supports the proposition that continuous fermentation, using immobilized yeast and the associated free cells in a gas-lift draft tube bioreactor system, is a viable alternative to batch fermentation for beer production based on the following criteria:

DETD [0892] higher bioreactor volumetric productivity

DETD . . . fermentation system technically feasible. The Grolsch brewery in the Netherlands has been reported to use a 230 m.sup.3 gas lift bioreactor for treatment of their wastewater (Driessen et al., 1997). One of the biggest barriers to commercial scale continuous fermentation in. . .

DETD . . . the fluidizing gas for beer flavour formation. The findings showed that under the given operating conditions, increased air in the bioreactor fluidizing gas caused an increase in acetaldehyde, diacetyl, and higher alcohols (isoamyl alcohol and isobutanol), while the concentrations of esters. . . ethanol were reduced. These data suggest that there is the potential for controlling beer flavour through the composition of the bioreactor fluidizing gas, allowing for the production of unique products.

. . the exception of when air was eliminated from the fluidizing DETD gas, a freely suspended cell concentration of greater than 10.sup.8 cells/mL was maintained in the bioreactor liquid phase. The system thus has more than one population of yeast cells coexisting in the bioreactor, the immobilized yeast and the liquid phase suspended yeast. Because of the large quantities of viable yeast growing in the bioreactor liquid phase, the possibility exists of using a continuous bioreactor as a yeast propagator. When a secondary 48-hour batch-holding period was added following continuous primary fermentation, a flavour profile within. . . the ideal scenario would be to entirely eliminate the secondary holding period by optimizing the conditions in the primary continuous bioreactor. However, further reductions in the secondary holding time can be achieved in the short term by optimizing the holding temperature.

DETD . . . more susceptible to microbial contamination (i.e. high sugar

concentration, temperature, and oxygen, with low concentrations of ethanol). In the gas-lift bioreactor presented in this work, the bioreactor has a low steady state fermentable sugar concentration, low pH, high ethanol concentration, and low concentrations of oxygen, making the. . .

DETD . . . showed an average diacetyl reduction of 46%. However, because ALDC is an enzyme that is produced by a genetically modified organism (GMO), there are public perception issues that would need to be addressed before using such an enzyme in a commercial. .

DETD [0904] Over six months of continuous fermentation using kappa-carrageenan gel immobilization, freely suspended cells in the liquid phase retained viabilities greater than 90%, while immobilized cell viability decreased to less than 60%. Scanning electron micrographs revealed that cells located near the periphery of the gel bead had multiple bud scars and a regular morphology, while those near the. . . in a plant environment, and, if the yeast flocs were disrupted on a regular basis, one could ensure that aged cells are regularly removed from the bioreactor.

CLM What is claimed is:

. . A process for the continuous fermentation of beer wherein a wort is at least partially fermented in a gas lift bioreactor employing a flocculent yeast strain with a restricted oxygen supply.

CLM What is claimed is:
8. The process according to claim 1 wherein the continuous stage is carried out in a gas lift bioreactor.

CLM What is claimed is:

9. The process according to claim 1 wherein said continuous fermentation stage employs "immobilized" cells selected from one of the group consisting of carrier immobilized yeast or flocculating yeasts.

=> s peristaltic and rollers and algae L14 36 PERISTALTIC AND ROLLERS AND ALGAE

=> s 114 and cultur?

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=> s 115 and flexible tubes

L16 5 L15 AND FLEXIBLE TUBES

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L16 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2010 THOMSON REUTERS on STN AN 2007-13331 BIOTECHDS

TI Culturing algae comprises placing algae in aqueous medium in a closed system bioreactor, exposing the algae to sunlight, and culturing the algae under conditions allowing algal reproduction and growth;

involving cell culture of alga in a photoreactor useful for diesel production

AU SEARS J T

PA SUNSOURCE IND

PI US 20070048848 1 Mar 2007

AI US 2006-510148 24 Aug 2006

PRAI US 2006-510148 24 Aug 2006; US 2005-711316 25 Aug 2005

DT Patent

LA English

OS WPI: 2007-387522 [36]

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L16 ANSWER 2 OF 5 IFIPAT COPYRIGHT 2010 IFI on STN
ΑN
      11398828 IFIPAT; IFIUDB; IFICDB
TΤ
      Method, apparatus and system for biodiesel production from algae
ΙN
      Sears James T
PA
      SUNSOURCE Ind
                          20070301 (CITED IN 001 LATER PATENTS)
PΤ
      US 20070048848 A1
ΑI
      US 2006-510148
                          20060824 (11)
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     US 2005-711316P
                           20050825 (Provisional)
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                           20060630 (Provisional)
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DT
      Utility; Patent Application - First Publication
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      CHEMICAL
      APPLICATION
      Entered STN: 5 Mar 2007
ED
      Last Updated on STN: 12 Apr 2007
CLMN
L16 ANSWER 3 OF 5 USPATFULL on STN
       2010:179163 USPATFULL
ΑN
ΤI
       PHOTOBIOREACTOR SYSTEMS
ΙN
       Schuring, Christopher S., Penryn, CA, UNITED STATES
       McCue, J. Kyle, San Jose, CA, UNITED STATES
PΙ
       US 20100159579
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ΑI
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       NCLM: 435/292.100
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       2007:55917 USPATFULL
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       Closed system bioreactor apparatus
ΤN
       Sears, James T., Boulder, CO, UNITED STATES
PA
       SUNSOURCE INDUSTRIES (U.S. corporation)
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       US 20070048859
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ΑI
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ΑN
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TΙ
       Method, apparatus and system for biodiesel production from algae
TM
       Sears, James T., Boulder, CO, UNITED STATES
       SUNSOURCE INDUSTRIES (U.S. corporation)
PA
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       INCLS: 435/257.100; 554/174.000
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